WEST Search History

DATE: Friday, June 13, 2003

Set Name side by side	Query	Hit Count	Set Name result set
DB=JPAB,EPAB,DWPI; PLUR=YES; OP=ADJ			
L9	L7 and @pD<19970421	3	L9
L8	L7 and @pd<19940819	0	L8
L7	L6 and ($arthritis or chodrodystophy or rheumatic or lupus or spondyl$ or herniated)	13	L7
L6	hepatocyte growth factor or hgf or scatter factor	483	L6
DB=USPT; PLUR=YES; OP=ADJ			
L5	l3 not L4	67	L5
L4	L3 and @ad<19940819	13	L4
L3	L2 and @AD<19970421	80	L3
L2	L1 and ($arthritis or chodrodystophy or rheumatic or lupus or spondyl$ or herniated)	274	L2
L1	hepatocyte growth factor or hgf or scatter factor	1028	L1

END OF SEARCH HISTORY

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* * * * * * * * * * * * * * STN  Columbus  * * * * * * * * * * * * * * * *

FILE 'HOME' ENTERED AT 10:58:44 ON 13 JUN 2003

=> file reg
COST IN U.S. DOLLARS                                    SINCE FILE      TOTAL
                                                           ENTRY    SESSION
FULL ESTIMATED COST                                         0.21       0.21

FILE 'REGISTRY' ENTERED AT 10:58:54 ON 13 JUN 2003

=> e hepatocyte growth factor/cn
E1            1      HEPATOCELLULAR CARCINOMA-RELATED PUTATIVE TUMOR SUPPRESSOR (
                     HUMAN LIVER GENE LPTS)/CN
E2            1      HEPATOCYTE GROWTH ACTOR ACTIVATOR PROTEIN (MELEAGRIS GALLOPA
                     VO PRECURSOR)/CN
E3            1 --> HEPATOCYTE GROWTH FACTOR/CN
E4            1      HEPATOCYTE GROWTH FACTOR (132-GLUTAMATE, 134-GLUTAMATE) (HUM
                     AN)/CN
E5            1      HEPATOCYTE GROWTH FACTOR (170-GLUTAMATE, 181-GLUTAMATE) (HUM
                     AN)/CN
E6            1      HEPATOCYTE GROWTH FACTOR (ALANINE-214) (HUMAN TRUNCATED ISOF
                     ORM NK2)/CN
E7            2      HEPATOCYTE GROWTH FACTOR (CAT CLONE PCI-FHGH)/CN
E8            1      HEPATOCYTE GROWTH FACTOR (CAT LIVER)/CN
E9            1      HEPATOCYTE GROWTH FACTOR (FELIS CATUS CLONE PCI-DFHGH DELETI
                     ON MUTANT)/CN
E10           1      HEPATOCYTE GROWTH FACTOR (FELIS CATUS CLONE PCI-FHGH)/CN
E11           4      HEPATOCYTE GROWTH FACTOR (HUMAN FRAGMENT) FUSION PROTEIN WIT
                     H MACROPHAGE-STIMULATING PROTEIN (HUMAN FRAGMENT)/CN
E12           1      HEPATOCYTE GROWTH FACTOR (HUMAN GENE HGF)/CN

=> s e3
L1            1 "HEPATOCYTE GROWTH FACTOR"/CN

=> d

L1   ANSWER 1 OF 1  REGISTRY  COPYRIGHT 2003 ACS
RN   67256-21-7  REGISTRY *
* Use of this CAS Registry Number alone as a search term in other STN files may
  result in incomplete search results.  For additional information, enter HELP
  RN* at an online arrow prompt (=>).
CN   Lymphokines and Cytokines, scatter factor  (CA INDEX NAME)
OTHER CA INDEX NAMES:
CN   Hepatocyte growth factor
OTHER NAMES:
CN   Animal growth regulators, epithelial scattering factors
CN   Animal growth regulators, hepatocyte growth factors
CN   Animal growth regulators, scatter factors
CN   Cytokines, scatter factor
CN   Hepatic stimulatory substance
CN   Hepatopoietin
CN   Hepatopoietin A
CN   Hepatotropic hormone
CN   Hepatotropin
CN   HGF
CN   Liver regenerative animal growth substances
DR   93196-17-9
MF   Unspecified
CI   MAN, CTS
LC   STN Files:   ADISNEWS, AGRICOLA, ANABSTR, BIOTECHNO, CA, CANCERLIT,
       CAPLUS, CHEMCATS, CIN, DRUGNL, DRUGUPDATES, EMBASE, MEDLINE, PHAR,
       TOXCENTER

*** STRUCTURE DIAGRAM IS NOT AVAILABLE ***
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            51 REFERENCES IN FILE CA (1957 TO DATE)
            51 REFERENCES IN FILE CAPLUS (1957 TO DATE)


=> set expand cont
SET COMMAND COMPLETED

=> sel l1 name
E13 THROUGH E25 ASSIGNED

=> index bioscience
FILE 'DRUGMONOG' ACCESS NOT AUTHORIZED
COST IN U.S. DOLLARS                                SINCE FILE      TOTAL
                                                       ENTRY    SESSION
FULL ESTIMATED COST                                     6.99       7.20

INDEX 'ADISCTI, ADISINSIGHT, ADISNEWS, AGRICOLA, ANABSTR, AQUASCI, BIOBUSINESS,
       BIOCOMMERCE, BIOSIS, BIOTECHABS, BIOTECHDS, BIOTECHNO, CABA, CANCERLIT,
       CAPLUS, CEABA-VTB, CEN, CIN, CONFSCI, CROPB, CROPU, DDFB, DDFU, DGENE,
       DRUGB, DRUGLAUNCH, DRUGMONOG2, ...' ENTERED AT 10:59:51 ON 13 JUN 2003

67 FILES IN THE FILE LIST IN STNINDEX

Enter SET DETAIL ON to see search term postings or to view
search error messages that display as 0* with SET DETAIL OFF.


=> s e13-25 and (osteoarthritis or arthritis or chondrodystrophy or rheumatic or
lupus or spondyl? or herniated)
          1   FILE ADISCTI
          1   FILE ADISNEWS
   3 FILES SEARCHED...
   6 FILES SEARCHED...
         17   FILE BIOSIS
   9 FILES SEARCHED...
          8   FILE BIOTECHABS
          8   FILE BIOTECHDS
          9   FILE BIOTECHNO
  12 FILES SEARCHED...
         11   FILE CANCERLIT
  14 FILES SEARCHED...
         40   FILE CAPLUS
  16 FILES SEARCHED...
  19 FILES SEARCHED...
  21 FILES SEARCHED...
          1   FILE DDFU
  23 FILES SEARCHED...
        354   FILE DGENE
  24 FILES SEARCHED...
  27 FILES SEARCHED...
          1   FILE DRUGU
  30 FILES SEARCHED...
          1   FILE EMBAL
         15   FILE EMBASE
  32 FILES SEARCHED...
          8   FILE ESBIOBASE
  33 FILES SEARCHED...
          3   FILE FEDRIP
  37 FILES SEARCHED...
         11   FILE IFIPAT
  41 FILES SEARCHED...
         10   FILE JICST-EPLUS
          1   FILE LIFESCI
  44 FILES SEARCHED...
         16   FILE MEDLINE
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  47 FILES SEARCHED...
  50 FILES SEARCHED...
         7   FILE PASCAL
  51 FILES SEARCHED...
         4   FILE PHIN
  57 FILES SEARCHED...
        28   FILE SCISEARCH
  59 FILES SEARCHED...
         4   FILE TOXCENTER
  61 FILES SEARCHED...
       756   FILE USPATFULL
  62 FILES SEARCHED...
        19   FILE USPAT2
  64 FILES SEARCHED...
        37   FILE WPIDS
  66 FILES SEARCHED...
        37   FILE WPINDEX

  27 FILES HAVE ONE OR MORE ANSWERS,    67 FILES SEARCHED IN STNINDEX

L2    QUE ("ANIMAL GROWTH REGULATORS, EPITHELIAL SCATTERING FACTORS"/BI OR "ANIM
          AL GROWTH REGULATORS, HEPATOCYTE GROWTH FACTORS"/BI OR "ANIMAL GROWTH
          REGULATORS, SCATTER FACTORS"/BI OR "CYTOKINES, SCATTER FACTOR"/BI OR "
          HEPATIC STIMULATORY SUBSTANCE"/BI OR "HEPATOCYTE GROWTH FACTOR"/BI OR
          "HEPATOPOIETIN A"/BI OR HEPATOPOIETIN/BI OR "HEPATOTROPIC HORMONE"/BI
          OR HEPATOTROPIN/BI OR HGF/BI OR "LIVER REGENERATIVE ANIMAL GROWTH SUBS
          TANCES"/BI OR "LYMPHOKINES AND CYTOKINES, SCATTER FACTOR"/BI) AND (OST
          EOARTHRITIS OR ARTHRITIS OR CHONDRODYSTROPHY OR RHEUMATIC OR LUPUS OR
          SPONDYL? OR HERNIATED)


=> s (scatter factor) and (osteoarthritis or arthritis or chondrodystrophy or
rheumatic or lupus or spondyl? or herniated)
         3   FILE BIOSIS
         1   FILE BIOTECHABS
         1   FILE BIOTECHDS
         8   FILE BIOTECHNO
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         3   FILE CANCERLIT
         3   FILE CAPLUS
       334   FILE DGENE
  30 FILES SEARCHED...
        17   FILE EMBASE
         1   FILE ESBIOBASE
         3   FILE MEDLINE
  46 FILES SEARCHED...
         1   FILE PASCAL
         1   FILE PROMT
         9   FILE SCISEARCH
       102   FILE USPATFULL
  62 FILES SEARCHED...
         1   FILE USPAT2
         7   FILE WPIDS
         7   FILE WPINDEX

  17 FILES HAVE ONE OR MORE ANSWERS,    67 FILES SEARCHED IN STNINDEX

L3    QUE (SCATTER FACTOR) AND (OSTEOARTHRITIS OR ARTHRITIS OR CHONDRODYSTROPHY
          OR RHEUMATIC OR LUPUS OR SPONDYL? OR HERNIATED)

=> s (l2 or l3) and py<1995
         1   FILE ADISCTI
         0*  FILE ADISINSIGHT
   3 FILES SEARCHED...
   4 FILES SEARCHED...
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    7 FILES SEARCHED...
            6   FILE BIOSIS
    9 FILES SEARCHED...
            3   FILE BIOTECHNO
   12 FILES SEARCHED...
   13 FILES SEARCHED...
            3   FILE CANCERLIT
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            3   FILE MEDLINE
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            1   FILE PASCAL
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            0*  FILE PHAR
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            3   FILE SCISEARCH
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   61 FILES SEARCHED...
            8   FILE USPATFULL
   62 FILES SEARCHED...
   64 FILES SEARCHED...
   66 FILES SEARCHED...

   11 FILES HAVE ONE OR MORE ANSWERS,   67 FILES SEARCHED IN STNINDEX

L4   QUE (L2 OR L3) AND PY<1995

=> d rank
F1            8   USPATFULL
F2            6   BIOSIS
F3            4   EMBASE
F4            3   BIOTECHNO
F5            3   CANCERLIT
F6            3   MEDLINE
F7            3   SCISEARCH
F8            1   ADISCTI
F9            1   CAPLUS
F10           1   ESBIOBASE
F11           1   PASCAL


=> file hits
COST IN U.S. DOLLARS                          SINCE FILE      TOTAL
                                                 ENTRY    SESSION
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FULL ESTIMATED COST                                  31.90      39.10

FILE 'USPATFULL' ENTERED AT 11:34:29 ON 13 JUN 2003
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=> s l4
   1 FILES SEARCHED...
   2 FILES SEARCHED...
   3 FILES SEARCHED...
   4 FILES SEARCHED...
   5 FILES SEARCHED...
   6 FILES SEARCHED...
   7 FILES SEARCHED...
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  10 FILES SEARCHED...
L5          34 L4

=> dup rem l5
PROCESSING COMPLETED FOR L5
L6           17 DUP REM L5 (17 DUPLICATES REMOVED)
                ANSWERS '1-8' FROM FILE USPATFULL
                ANSWERS '9-14' FROM FILE BIOSIS
                ANSWERS '15-17' FROM FILE EMBASE

=> d bib abs 1-17

L6   ANSWER 1 OF 17  USPATFULL
AN     1999:4861  USPATFULL
TI     Human basic fibroblast growth factor analog
IN     Fiddes, John C., 2320 Bryant St., Palo Alto, CA, United States  94301
       Abraham, Judith A., 655 S. Fairoaks Ave., Sunnyvale, CA, United States
       94086
```

Protter, Andrew A., 185 N. California Ave., Palo Alto, CA, United States 94301
PI US 5859208 19990112
WO 8900198 19890112 <--
AI US 1990-459739 19900212 (7)
WO 1988-US2264 19880706
19900212 PCT 371 date
19900212 PCT 102(e) date
DT Utility
FS Granted
EXNAM Primary Examiner: Walsh, Stephen
LREP Lehnhardt, Susan K.
CLMN Number of Claims: 1
ECL Exemplary Claim: 1
DRWN 8 Drawing Figure(s); 8 Drawing Page(s)
LN.CNT 2048
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB A human basic fibroblast growth factor (FGF) protein analog wherein the cysteine at positions 78 and 96 is replaced by serine, and said analog exhibits the biological activity of native, human basic FGF.

The DNA sequences encoding analogs of human acidic and basic fibroblast growth factors (FGF) can be recombinantly expressed to obtain practical amounts of proteins useful in effecting both pathologies related to persistent angiogenesis and wound healing and related tissue repair.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L6 ANSWER 2 OF 17 USPATFULL
AN 94:93413 USPATFULL
TI Cysteine depleted IL-6 muteins
IN Skelly, Susan M., Dunellen, NJ, United States
Tackney, Charles T., Brooklyn, NY, United States
Snouwaert, John N., Carrboro, NC, United States
Fowlkes, Dana M., Chapel Hill, NC, United States
PA Imclone Systems Inc., New York, NY, United States (U.S. corporation)
The University of North Carolina at Chapel Hill, Chapel Hill, NC, United States (U.S. corporation)
PI US 5359034 19941025 <--
AI US 1992-907710 19920702 (7)
RLI Continuation-in-part of Ser. No. US 1991-724698, filed on 2 Jul 1991, now abandoned
DT Utility
FS Granted
EXNAM Primary Examiner: Draper, Garnette D.
LREP Feit, Irving N., Weiss, Laura S.
CLMN Number of Claims: 10
ECL Exemplary Claim: 1
DRWN 18 Drawing Figure(s); 18 Drawing Page(s)
LN.CNT 1651
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB Muteins of IL-6 and truncated IL-6 are prepared by recombinant DNA techniques. In the muteins, the cysteine residues that occur at positions, or at positions corresponding to positions, 45 and 51 of mature, native IL-6 have been replaced by other amino acids. The cysteine residues that occur at positions, or at positions corresponding to positions, 74 and 84 are retained. The molecule has biological activity that is at least comparable to that of native IL-6.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L6 ANSWER 3 OF 17 USPATFULL
AN 93:100656 USPATFULL
TI Three-dimensional skin culture system
IN Naughton, Gail K., Groton, VT, United States

Naughton, Brian A., Groton, VT, United States
PA Advanced Tissue Sciences, Inc., La Jolla, CA, United States (U.S. corporation)
PI US 5266480 19931130 <--
AI US 1990-575518 19900830 (7)
RLI Division of Ser. No. US 1989-402104, filed on 1 Sep 1989, now patented, Pat. No. US 5032508 which is a continuation-in-part of Ser. No. US 1988-242096, filed on 8 Sep 1988, now patented, Pat. No. US 4963489 which is a continuation-in-part of Ser. No. US 1987-38110, filed on 14 Apr 1987, now abandoned which is a continuation-in-part of Ser. No. US 1987-36154, filed on 3 Apr 1987, now patented, Pat. No. US 4721096 which is a continuation of Ser. No. US 1986-853569, filed on 18 Apr 1986, now abandoned
DT Utility
FS Granted
EXNAM Primary Examiner: Wityshyn, Michael G.; Assistant Examiner: Williams, Jane A.
LREP Pennie & Edmonds
CLMN Number of Claims: 30
ECL Exemplary Claim: 1
DRWN 25 Drawing Figure(s); 25 Drawing Page(s)
LN.CNT 3115
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The present invention relates to a three-dimensional cell culture system which can be used to culture a variety of different cells and tissues in vitro for prolonged periods of time. In accordance with the invention, cells derived from a desired tissue are inoculated and grown on a pre-established stromal support matrix. The stromal support matrix comprises stromal cells, such as fibroblasts actively growing on a three-dimensional matrix. Stromal cells may also include other cells found in loose connective tissue such as endothelial cells, macrophages/monocytes, adipocytes, pericytes, reticular cells found in bone marrow stroma, etc. The stromal matrix provides the support, growth factors, and regulatory factors necessary to sustain long-term active proliferation of cells in culture. When grown in this three-dimensional system, the proliferating cells mature and segregate properly to form components of adult tissues analogous to counterparts found in vivo.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L6 ANSWER 4 OF 17 USPATFULL
AN 93:56844 USPATFULL
TI DNA encoding platelet derived endothelial cell growth factor (PD-ECGF)
IN Heldin, Carl-Henrik, Uppsala, Sweden
Miyazono, Kohei, Tokyo, Japan
Wernstedt, Christer, Uppsala, Sweden
Hellman, Ulf G. T., Uppsala, Sweden
Takaku, Fumimaro, Tokyo, Japan
Ishikawa, Fuyuki, Tokyo, Japan
PA Ludwig Institute for Cancer Research, NY, United States (U.S. corporation)
PI US 5227302 19930713 <--
AI US 1991-742092 19910802 (7)
RLI Continuation of Ser. No. US 1988-288056, filed on 20 Dec 1988, now abandoned
DT Utility
FS Granted
EXNAM Primary Examiner: Hill, Jr., Robert J.; Assistant Examiner: Allen, Marianne Porta
LREP Felfe & Lynch
CLMN Number of Claims: 11
ECL Exemplary Claim: 1
DRWN 35 Drawing Figure(s); 26 Drawing Page(s)
LN.CNT 2038
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB Platelet-derived endothelial cell growth factor (PD-ECGF) is a 45 kDa endothelial cell mitogen that has been purified to homogeneity from human platelets. It does not bind to heparin and does not stimulate the proliferation of fibroblasts, in contrast to other endothelial mitogens of the fibroblast growth factor (FGF) family. PD-ECGF appears to be the only endothelial cell growth factor in human platelets and recent data indicate that it has angiogenic activity in vitro, i.e., the ability to stimulate the formation of new blood vessels and chemotactic activity, in vitro. The present invention provides a homogeneous PD-ECGF in substantially greater yields than available in the past, the primary structure of PD-ECGF, antibodies against PD-ECGF, clones of its cDNA, and variants thereof. The invention also provides a therapeutic preparation of PD-ECGF.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L6 ANSWER 5 OF 17 USPATFULL
AN 92:90776 USPATFULL
TI Three-dimensional cell and tissue culture apparatus
IN Naughton, Gail K., Groton, VT, United States
Naughton, Brian A., Groton, VT, United States
PA Marrow-Tech Incorporated, La Jolla, CA, United States (U.S. corporation)
PI US 5160490 19921103 <--
AI US 1991-659220 19910221 (7)
RLI Division of Ser. No. US 1989-402104, filed on 1 Sep 1989, now patented, Pat. No. US 5032508 which is a continuation-in-part of Ser. No. US 1988-242096, filed on 8 Sep 1988, now patented, Pat. No. US 4963489, issued on 16 Oct 1990 which is a continuation-in-part of Ser. No. US 1987-38110, filed on 14 Apr 1987, now abandoned which is a continuation-in-part of Ser. No. US 1987-36154, filed on 3 Apr 1987, now patented, Pat. No. US 4721096, issued on 26 Jan 1988 which is a continuation of Ser. No. US 1986-853569, filed on 18 Apr 1986, now abandoned
DT Utility
FS Granted
EXNAM Primary Examiner: Robinson, Douglas W.; Assistant Examiner: Williams, Jane A.
LREP Pennie & Edmonds
CLMN Number of Claims: 19
ECL Exemplary Claim: 1
DRWN 25 Drawing Figure(s); 25 Drawing Page(s)
LN.CNT 3060
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The present invention relates to a three-dimensional cell culture system which can be used to culture a variety of different cells and tissues in vitro for prolonged periods of time. In accordance with the invention, cells derived from a desired tissue are inoculated and grown on a pre-established stromal support matrix. The stromal support matrix comprises stromal cells, such as fibroblasts actively growing on a three-dimensional matrix. Stromal cells may also include other cells found in loose connective tissue such as endothelial cells, macrophages/monocytes, adipocytes, pericytes, reticular cells found in bone marrow stroma, etc. The stromal matrix provides the support, growth factors, and regulatory factors necessary to sustain long-term active proliferation of cells in culture. When grown in this three-dimensional system, the proliferating cells mature and segregate properly to form components of adult tissues analogous to counterparts found in vivo.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L6 ANSWER 6 OF 17 USPATFULL
AN 92:86961 USPATFULL
TI Method for prophylaxis of obesity
IN Schwartz, Arthur G., Philadelphia, PA, United States
Lewbart, Marvin L., Media, PA, United States

PA Research Corporation Technologies, Inc., Tuscon, AZ, United States (U.S. corporation)
PI US 5157031 19921020 <--
AI US 1989-326355 19890321 (7)
RLI Continuation of Ser. No. US 1986-867112, filed on 21 May 1986, now abandoned which is a continuation-in-part of Ser. No. US 1983-519550, filed on 2 Aug 1983, now abandoned
DT Utility
FS Granted
EXNAM Primary Examiner: Rizzo, Nicholas S.
LREP Scully, Scott, Murphy & Presser
CLMN Number of Claims: 19
ECL Exemplary Claim: 1
DRWN No Drawings
LN.CNT 1163
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L6 ANSWER 7 OF 17 USPATFULL
AN 91:56847 USPATFULL
TI Three-dimensional cell and tissue culture system
IN Naughton, Gail K., Groton, VT, United States
Naughton, Brian A., Groton, VT, United States
PA Marrow-Tech, Inc., La Jolla, CA, United States (U.S. corporation)
PI US 5032508 19910716 <--
AI US 1989-402104 19890901 (7)
RLI Continuation-in-part of Ser. No. US 1988-242096, filed on 8 Sep 1988, now patented, Pat. No. US 4963489 which is a continuation-in-part of Ser. No. US 1987-38110, filed on 14 Apr 1987, now abandoned which is a continuation-in-part of Ser. No. US 1987-36154, filed on 3 Apr 1987, now patented, Pat. No. US 4721096 which is a continuation of Ser. No. US 1986-853569, filed on 18 Apr 1986, now abandoned
DT Utility
FS Granted
EXNAM Primary Examiner: Kepplinger, Esther L.; Assistant Examiner: Scheiner, Toni
LREP Pennie & Edmonds
CLMN Number of Claims: 19
ECL Exemplary Claim: 1
DRWN 25 Drawing Figure(s); 25 Drawing Page(s)
LN.CNT 2971
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The present invention relates to a three-dimensional cell culture system which can be used to culture a variety of different cells and tissues in vitro for prolonged periods of time. In accordance with the invention, cells derived from a desired tissue are inoculated and grown on a pre-established stromal support matrix. The stromal support matrix comprises stromal cells, such as fibroblasts actively growing on a three-dimensional matrix. Stromal cells may also include other cells found in loose connective tissue such as endothelial cells, macrophages/monocytes, adipocytes, pericytes, reticular cells found in bone marrow stroma, etc. The stromal matrix provides the support, growth factors, and regulatory factors necessary to sustain long-term active proliferation of cells in culture. When grown in this three-dimensional system, the proliferating cells mature and segregate properly to form components of adult tissues analogous to counterparts found in vivo.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L6 ANSWER 8 OF 17 USPATFULL
AN 90:79819 USPATFULL
TI Three-dimensional cell and tissue culture system
IN Naughton, Gail K., Groton, VT, United States
Naughton, Brian A., Groton, VT, United States
PA Marrow-Tech, Inc., La Jolla, CA, United States (U.S. corporation)

PI US 4963489 19901016 <--
AI US 1988-242096 19880908 (7)
RLI Continuation-in-part of Ser. No. US 1987-38110, filed on 14 Apr 1987 which is a continuation-in-part of Ser. No. US 1987-36154, filed on 3 Apr 1987, now patented, Pat. No. US 4721096 which is a continuation of Ser. No. US 1986-853569, filed on 18 Apr 1986, now abandoned
DT Utility
FS Granted
EXNAM Primary Examiner: Warren, Charles F.; Assistant Examiner: Low, Christopher
LREP Pennie & Edmonds
CLMN Number of Claims: 10
ECL Exemplary Claim: 1
DRWN 10 Drawing Figure(s); 8 Drawing Page(s)
LN.CNT 2118
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The present invention relates to a three-dimensional cell culture system which can be used to culture a variety of different cells and tissues in vitro for prolonged periods of time. In accordance with the invention, cells derived from a desired tissue are inoculated and grown on a pre-established stromal support matrix. The stromal support matrix comprises stromal cells, such as fibroblasts, grown to subconfluence on a three-dimensional matrix. Stromal cells may also include other cells found in loose connective tissue such as endothelial cells, macrophages/monocytes, adipocytes, pericytes, reticular cells found in bone marrow stroma, etc. The stromal matrix provides the support, growth factors, and regulatory factors necessary to sustain long-term active proliferation of cells in culture. When grown in this three-dimensional system, the proliferating cells mature and segregate properly to form components of adult tissues analogous to counterparts found in vivo.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L6 ANSWER 9 OF 17 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.DUPLICATE 1
AN 1995:78707 BIOSIS
DN PREV199598093007
TI Levels of **hepatocyte growth factor** in synovial fluid and serum of patients with rheumatoid **arthritis** and release of **hepatocyte growth factor** by rheumatoid synovial fluid cells.
AU Yukioka, Kazuhiko (1); Inaba, Masaaki; Furumitsu, Yutaka; Yukioka, Masao; Nishino, Tomoyoshi; Goto, Hitoshi; Nishizawa, Yoshiki; Morii, Hirotoshi
CS (1) Yukioka Hosp., 2-2-3, Ukita-cho, Kita-ku, Osaka City 530 Japan
SO Journal of Rheumatology, (1994) Vol. 21, No. 12, pp. 2184-2189. ISSN: 0315-162X.
DT Article
LA English
AB Objective. To determine the levels of **hepatocyte growth factor** (**HGF**) in synovial fluids (SF) and sera from patients with rheumatoid **arthritis** (RA); to examine how these correlate with several disease variables in patients with RA and with levels of interleukin-6 (IL-6) in SF of these patients; and to examine whether **HGF** is released from adherent synovial cells (ASC) and synovial fluid cells (SF cells). Methods. An enzyme linked immunosorbent assay was used to measure levels of **HGF** and IL-6. SF samples were obtained from 22 patients with PA, 12 with **osteoarthritis** (OA), and one with septic **arthritis**. Serum samples were collected from 40 patients with RA. **HGF** levels in culture supernatants from ASC and SF cells were measured. Results. The mean values of **HGF** in SF were 1.21 ng/ml for patients with RA, 0. 19 ng/ml for those with OA and 0. 18 ng/ml for the one with septic **arthritis**. **HGF** levels in SF of patients with RA were significantly higher than of those with OA (p lt 0.01). The levels for patients with RA correlated with the serum C-reactive protein

concentrations (r = 0.626, p lt 0.01) and IL-6 levels in SF (r = 0.476, p lt 0.05). The mean value of **HGF** in sera from patients with RA was 0.28 ng/ml. **HGF** levels in SF were higher than those in sera drawn simultaneously from the same patients with RA. In vitro, release of **HGF** from rheumatoid ASC was not detected. However, SF cells from patients with RA released **HGF** spontaneously. Conclusion. Our observations suggest that **HGF** in SF of patients with RA is produced by SF cells and is related to disease activity of RA, and thus that **HGF** may play a role in RA.

L6 ANSWER 10 OF 17 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.DUPLICATE 2
AN 1994:514671 BIOSIS
DN PREV199497527671
TI **Hepatocyte growth factor/scatter factor**: A cytokine mediating endothelial migration in rheumatoid **arthritis**.
AU Koch, A. E. (1); Halloran, M.; Hosaka, S.; Shah, M. R.; Haskell, C. J.; Baker, S. K.; Panos, R. J.; Haines, G. K.; Bennett, G.; Schwall, R.; Pope, R. M.; Ferrara, N.
CS (1) Dep. Med., Northwestern Univ., Chicago, IL 60611 USA
SO Clinical Research, (1994) Vol. 42, No. 3, pp. 385A.
Meeting Info.: Combined Annual Meeting of the Central Society for Clinical Research, American Federation for Clinical Research, Midwest Section, Midwest Society for Pediatric Research, Society for Investigative Dermatology, Central Region, and the Midwest Society of General Internal Medicine Chicago, Illinois, USA September 16-18, 1994
ISSN: 0009-9279.
DT Conference
LA English

L6 ANSWER 11 OF 17 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.DUPLICATE 4
AN 1989:313169 BIOSIS
DN BA88:26899
TI INTERLEUKIN-6 IL-6 AND ACUTE PHASE PROTEINS IN THE DISEASE COURSE OF PATIENTS WITH SYSTEMIC **LUPUS** ERYTHEMATOSUS.
AU SWAAK A J G; VAN ROOYEN A; AARDEN L A
CS DEP. RHEUMATOL., DR. DANIEL DEN HOED CLINIC, GROENE HILLEDIJK 301, NL-3075 EA ROTTERDAM, NETHERLANDS.
SO RHEUMATOL INT, (1989) 8 (6), 263-268.
CODEN: RHINDE. ISSN: 0172-8172.
FS BA; OLD
LA English
AB One of the mediators responsible for the induction of the production of acute phase proteins by hepatocytes is interleukin-6 (IL-6), formally known as hybridoma growth factor (**HGF**). In a prospective study the biological significance of IL-6, but also the relationship with the acute phase response (C-reactive protein [CRP], .alpha.1-antitrypsin and .alpha.1-acid glycoprotein) during flare-ups in 12 systemic **lupus** erythematosus (SLE) patients was investigated. Only 2 SLE patients showed sustained elevated IL-6 levels, and in one of these patients a clear correlation was found between the increases in IL-6 and the acute phase response. In the other SLE patients hardly any response or change in the levels of IL-6, CRP, and/or .alpha.1-antitrypsin was found. In contrast to the profiles of .alpha.1-acid glycoprotein, in seven of the SLE patients a significant increase in the serum levels took place in the period preceding the exacerbation. The difference between the three acute phase proteins suggests that the regulatory mechanisms are different. Our results are in agreement with the findings that IL-6 might be responsible for the CRP response.

L6 ANSWER 12 OF 17 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
AN 1994:443725 BIOSIS
DN PREV199497456725

TI Blood **hepatocyte growth factor** levels in chronic renal failure patients.
AU Chang, H. (1); Nagao, T.; Ichikawa, N.; Kawamoto, T.; Nakamura, T.; Kurokawa, K.; Asano, S.; Katoh, T.
CS (1) Fourth Dep. Internal Med., Univ. Tokyo Sch. Med., 3-28-6 Meijirodai, Bunkyo-ku, Tokyo 112 Japan
SO Nephron, (1994) Vol. 67, No. 4, pp. 497-498.
ISSN: 0028-2766.
DT Article; Letter
LA English

L6 ANSWER 13 OF 17 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
AN 1995:3862 BIOSIS
DN PREV199598018162
TI The possible important role of **hepatocyte growth factor** in the development of rheumatoid **arthritis**.
AU Goto, Hitoshi (1); Hino, Masayuki; Inaba, Masaaki; Nishizawa, Yoshiki; Morii, Hirotoshi; Yukioka, Kazuhiko; Furumitsu, Yutaka; Yukioka, Masao; Nishino, Tomoyoshi
CS (1) Osaka City Univ., Yukioka Hosp., Osaka Japan
SO Arthritis & Rheumatism, (1994) Vol. 37, No. 9 SUPPL., pp. S195.
Meeting Info.: 58th National Scientific Meeting of the American College of Rheumatology and the 29th National Scientific Meeting of the Association of Rheumatology Health Professionals Minneapolis, Minnesota, USA October 23-27, 1994
ISSN: 0004-3591.
DT Conference
LA English

L6 ANSWER 14 OF 17 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
AN 1995:3860 BIOSIS
DN PREV199598018160
TI **Hepatocyte growth factor**: A cytokine mediating endothelial migration in rheumatoid **arthritis**.
AU Koch, Alisa E. (1); Halloran, Margaret M.; Hosaka, Shigeru; Shah, Manisha R.; Haskell, Catherine J.; Baker, Steven K.; Panos, Ralph J.; Haines, G. Kenneth; Pope, Richard M.; Ferrara, Napoleone
CS (1) Dep. Med., Northwestern Univ., Chicago, IL 60611 USA
SO Arthritis & Rheumatism, (1994) Vol. 37, No. 9 SUPPL., pp. S195.
Meeting Info.: 58th National Scientific Meeting of the American College of Rheumatology and the 29th National Scientific Meeting of the Association of Rheumatology Health Professionals Minneapolis, Minnesota, USA October 23-27, 1994
ISSN: 0004-3591.
DT Conference
LA English

L6 ANSWER 15 OF 17 EMBASE COPYRIGHT 2003 ELSEVIER SCI. B.V.DUPLICATE 3
AN 92319477 EMBASE
DN 1992319477
TI Serum interleukin-6 levels and joint involvement in polyarticular and pauciarticular juvenile chronic **arthritis**.
AU De Benedetti F.; Robbioni P.; Massa M.; Viola S.; Albani S.; Martini A.
CS Clinica Pediatrica, IRCCS Policlinico S. Matteo, Piazzale Golgi 2,27100 Pavia, Italy
SO Clinical and Experimental Rheumatology, (1992) 10/5 (493-498).
ISSN: 0392-856X CODEN: CERHDP
CY Italy
DT Journal; Article
FS 007 Pediatrics and Pediatric Surgery
026 Immunology, Serology and Transplantation
031 Arthritis and Rheumatism
LA English
SL English
AB We measured serum and synovial fluid interleukin 6 (IL-6) levels in

patients with polyarticular-onset and pauciarticular-onset juvenile chronic **arthritis** (JCA), using the hybridoma cell line B9. During active disease, but not during remission, serum IL-6 levels were significantly elevated in patients with polyarticular ($p < 0.0001$ vs controls) and in patients with pauciarticular JCA ($p < 0.01$ vs controls). In patients with active polyarticular disease (polyarticular and extended pauciarticular) serum IL-6 levels correlated with the extent and severity of joint involvement ($p < 0.05$), with erythrocyte sedimentation rate values ($p < 0.005$) and with C reactive protein concentrations ($p < 0.05$). In patients with persistent pauciarticular JCA, serum IL-6 levels correlated with the joint swelling score ($p < 0.05$). Synovial fluid IL-6 levels, measured in 5 patients with pauciarticular JCA, were markedly elevated (range 600-24,000 **HGF** U/ml). In conclusion, our data suggest that IL-6 is an important pathogenic mediator in polyarticular and pauciarticular JCA.

L6 ANSWER 16 OF 17 EMBASE COPYRIGHT 2003 ELSEVIER SCI. B.V.DUPLICATE 5
AN 89103523 EMBASE
DN 1989103523
TI Interleukin-6 (IL-6) and acute phase proteins in the disease course of patients with systemic **lupus** erythematosus.
AU Swaak A.J.G.; Van Rooyen A.; Aarden L.A.
CS Department of Rheumatology, The Dr Daniel den Hoed Clinic, 3075 EA Rotterdam, Netherlands
SO Rheumatology International, (1988) 8/6 (263-268).
ISSN: 0172-8172 CODEN: RHINDE
CY Germany
DT Journal
FS 031 Arthritis and Rheumatism
LA English
SL English
AB One of the mediators responsible for the induction of the production of acute phase proteins by hepatocytes is interleukin-6 (IL-6), formally known as hybridoma growth factor (**HGF**). In a prospective study the biological significance of IL-6, but also the relationship with the acute phase response (C-reactive protein [CRP], .alpha.1-antitrypsin and .alpha.1-acid glycoprotein) during flare-ups in 12 systemic **lupus** erythematosus (SLE) patients was investigated. Only 2 SLE patients showed sustained elevated IL-6 levels, and in one of these patients a clear correlation was found between the increases in IL-6 and the acute phase response. In the other SLE patients hardly any response or change in the levels of IL-6, CRP, and/or .alpha.1-antitrypsin was found. In contrast to the profiles of .alpha.1 acid glycoprotein, in seven of the SLE patients a significant increase in the serum levels took place in the period preceding the exacerbation. This difference between the three acute phase proteins suggests that the regulatory mechanisms are different. Our results are in agreement with the findings that IL-6 might be responsible for the CRP response.

L6 ANSWER 17 OF 17 EMBASE COPYRIGHT 2003 ELSEVIER SCI. B.V.
AN 88013937 EMBASE
DN 1988013937
TI Autoimmune conditions induced by epithelial solid tumors.
AU Bataille R.; Klein B.
CS Immuno-Rhumatologie, Centre Gui-de-Chauliac, Hopital Saint-Eloi, F-34059 Montpellier Cedex, France
SO Rheumatology International, (1987) 7/6 (231-233).
ISSN: 0172-8172 CODEN: RHINDE
CY Germany
DT Journal
FS 006 Internal Medicine
018 Cardiovascular Diseases and Cardiovascular Surgery
031 Arthritis and Rheumatism
LA English
SL English

AB Numerous immunological and genetic factors are involved in the genesis of autoimmune diseases. It has recently been demonstrated that epithelial cells from cardiac myxomas, and also from some carcinomas, constitutively produce BSF-p2/IFN.beta.2/**HGF**, which is able to induce symptoms and autoantibodies suggestive of connective-tissue disease. These data are similar to what is observed in some murine **lupus** strains in which increased production of BCDF is responsible for an early severe SLE (i.e., MRL/lpr/lpr). Overproduction and/or constitutive production of BCDF has been demonstrated in patients with Kawasaki's disease, SLE and RA. Considering the nature of the bioassay used to study patients with SLE and RA, we can conclude that, at least in these diseases, the BCDF detected is identical to BSF-p2/IFN.beta.2/**HGF**. This factor could therefore be involved in Kawasaki's disease and other autoimmune diseases. Further investigations could be of interest, especially to see whether overproduction of this factor is an immune feature of DR3-positive normal individuals. Taking autoimmune states induced by epithelial solid tumors as a simple example of autoimmunity in humans, we suggest that BSF-p2/IFN.beta.2/**HGF** could play a major role in human autoimmune diseases by inducing a state of polyclonal B-cell hyperactivation.

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FULL ESTIMATED COST	147.88	186.98

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COST IN U.S. DOLLARS	SINCE FILE ENTRY	TOTAL SESSION
FULL ESTIMATED COST	0.36	187.34

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L9: Entry 2 of 3 File: DWPI Feb 29, 1996

DERWENT-ACC-NO: 1996-151144
DERWENT-WEEK: 200210

TITLE: Cartilage disease remedy contg. human growth factor - useful in treatment of various forms of arthritis such as acute suppurative arthritis, rheumatoid arthritis, etc.

INVENTOR: IWAMOTO, M; NAKAMURA, T ; NOJI, S

PATENT-ASSIGNEE: SUMITOMO SEIYAKU KK (SUMU), SUMITOMO PHARM CO LTD (SUMU)

PRIORITY-DATA: 1994JP-0218164 (August 19, 1994)

PATENT-FAMILY:

PUB-NO	PUB-DATE	LANGUAGE	PAGES	MAIN-IPC
WO 9605855 A1	February 29, 1996	J	030	A61K038/18
US 20020009432 A1	January 24, 2002		000	A61K048/00
JP 08059502 A	March 5, 1996		011	A61K038/22

DESIGNATED-STATES: CA US

CITED-DOCUMENTS:CA 2061211; EP 499242 ; JP 5132426 ; JP 6025010 ; JP 6172207

APPLICATION-DATA:

PUB-NO	APPL-DATE	APPL-NO	DESCRIPTOR
WO 9605855A1	January 30, 1995	1995WO-JP00121	
US20020009432A1	January 30, 1995	1995WO-JP00121	Cont of
US20020009432A1	April 21, 1997	1997US-0793121	Cont of
US20020009432A1	August 6, 2001	2001US-0921874	
JP 08059502A	August 19, 1994	1994JP-0218164	

INT-CL (IPC): A61 K 38/18; A61 K 38/22; A61 K 48/00; C07 K 14/475

ABSTRACTED-PUB-NO: US20020009432A
BASIC-ABSTRACT:

The following are claimed: (A) a cartilage disease remedy contg. hepatocyte growth factor (HGF) as the active ingredient; (B) a cartilage cell growth promoter contg. HGF as the active ingredient; and (C) a proteoglycan growth promoter contg. HGF as the active ingredient.

USE - HGF is useful in the treatment of deformed arthritis, cartilage formation disorder, cure and recovery of broken bones, recovery of joint cartilage and articular discs due to injury, acute suppurative arthritis, tuberculous arthritis, syphilitic arthritis, chronic rheumatoid arthritis, rheumatic fever, systemic lupus erythematosous, deformed myelopathy, hernia and recovery of bone transplant (claimed). HGF is administered in a dosage of 0.05-500 (pref. 1-100) mg/day.

ADVANTAGE - Using HGF results in less side effects.

ABSTRACTED-PUB-NO: WO 9605855A

EQUIVALENT-ABSTRACTS:

The following are claimed: (A) a cartilage disease remedy contg. hepatocyte growth factor (HGF) as the active ingredient; (B) a cartilage cell growth promoter contg. HGF as the active ingredient; and (C) a proteoglycan growth promoter contg. HGF as the active ingredient.

USE - HGF is useful in the treatment of deformed arthritis, cartilage formation disorder, cure and recovery of broken bones, recovery of joint cartilage and articular discs due to injury, acute suppurative arthritis, tuberculous arthritis, syphilitic arthritis, chronic rheumatoid arthritis, rheumatic fever, systemic lupus erythematosous, deformed myelopathy, hernia and recovery of bone transplant (claimed). HGF is administered in a dosage of 0.05-500 (pref. 1-100) mg/day.

ADVANTAGE - Using HGF results in less side effects.

CHOSEN-DRAWING: Dwg.0/8

DERWENT-CLASS: B04
CPI-CODES: B04-H06K; B14-C09; B14-G02D; B14-L06; B14-N01;

PATENT ABSTRACTS OF JAPAN

(11)Publication number : 08-059502

(43)Date of publication of application : 05.03.1996

(51)Int.Cl. A61K 38/22
A61K 38/22
A61K 38/22

(21)Application number : 06-218164

(22)Date of filing : 19.08.1994

(71)Applicant : SUMITOMO PHARMACEUT CO LTD

(72)Inventor : IWAMOTO YOUTAI
NOJI SUMIHARU
NAKAMURA TOSHIICHI

(54) AGENT FOR TREATING CARTILAGINOUS DISORDER

(57)Abstract:

PURPOSE: To obtain a cartilaginous disorder-treating agent having an action for stimulating the multiplication of cartilaginous cells and for stimulating the growth of proteoglucan and useful for treating and preventing various diseases caused by the cartilaginous disorder.

CONSTITUTION: The cartilaginous cell-treating agent, cartilaginous cell multiplication-stimulating agent and proteoglucan production-stimulating agent contains HGF(hepatocyte growth factor) as an active ingredient. The method for preparing the HGF includes a method for extracting the HGF front the rcrgans, hemocytes, plasma, serum, etc., of a mammalian and subsequently purifying the extracted HGF, a method for culturing the primary culture cells or established cells producing the HGF, separating the HGF from the culture product (the culture supernatant, the cultured cells, etc.) and subsequently purfiying the separated HGF, and a method for integrating a gene coding the HGF with a proper vector by a genetic optical means, inserting the product into a proper host, culturing the transformant and subsequently separating the HGF from the culture supernatant. The HGF is suitably administered at a dose of 0.05-500mg, preferably 1-100mg, once to several times a day.

LEGAL STATUS

[Date of request for examination] 16.08.2001

[Date of sending the examiner's decision of rejection]

[Kind of final disposal of application other than the examiner's decision of rejection or application converted registration]

[Date of final disposal for application]

[Patent number]

[Date of registration]

[Number of appeal against examiner's decision of rejection]

[Date of requesting appeal against examiner's decision of rejection]

[Date of extinction of right]

JAPANESE [JP,08-059502,A]

CLAIMS DETAILED DESCRIPTION TECHNICAL FIELD PRIOR ART EFFECT OF THE INVENTION TECHNICAL PROBLEM MEANS EXAMPLE DESCRIPTION OF DRAWINGS DRAWINGS

[Translation done.]

* NOTICES *

1. This document has been translated by computer.So the translation may not reflect the original precisely.
2. **** shows the word which can not be translated.
3.In the drawings, any words are not translated.

CLAIMS

[Claim(s)]
[Claim 1] The cartilage obstacle medical treatment agent characterized by containing HGF as an active principle.
[Claim 2] The chondrocyte multiplication accelerator characterized by containing HGF as an active principle.
[Claim 3] The proteoglycan generation accelerator characterized by containing HGF as an active principle.

[Translation done.]

DETAILED DESCRIPTION

[Detailed Description of the Invention]
[0001]
[Industrial Application] this invention relates to the cartilage-disorder treatment agent, chondrocyte proliferation accelerator, and proteoglycan generation accelerator which contain HGF (Hepatocyte Growth Factor) as an active principle in a detail more about a medicine useful to the treatment and prevention of a chondropathy.
[0002]
[Description of the Prior Art] A cartilage is a connective tissue which consists of a substrate which encloses a chondrocyte and this, and exists in a joint, the intervertebral disk of a spine, a costal cartilage, a pinna, external auditory meatus, pubic symphysis, a throat lid, etc. A cartilage consists of cartilage matrix which a chondrocyte and a chondrocyte produce, cartilage matrix is the components with main fiber component, proteoglycan, and water, such as a collagen fiber, and a cartilage can be classified into hyaline cartilage (a costal cartilage, a throat cartilage, articular cartilage, etc.), elastic cartilages (conchal cartilage etc.), and fibrocartilage (an intervertebral disk cartilage, a pubis cartilage, articular cartilage, etc.) according to mixture ****** of the cartilage matrix. In the above-mentioned cartilage matrix, a collagen fiber participates in the rigidity and intensity to the tension and shearing force of a cartilage, a proteoglycan participates in the intensity to compressive force, it is supposed that water is kept in the property as a viscoelastic body as a body tissue, for example, in the case of the articular cartilage, water occupied 78.6% of the nature weight of a cartilage, the collagen occupied 20%, and the proteoglycan occupies 7%. As an operation of a cartilage, reduction (cartilage between bones) of friction of epiphysis, maintenance (conchal cartilage etc.) of elasticity, and motor functions (a costal cartilage, pubis cartilage, etc.) are mentioned.
[0003]
[Problem(s) to be Solved by the Invention] As mentioned above, the cartilage has the important operation, after that a living body maintains [functional], the various disorders which originate in the obstacle of a cartilage from the former are known, for example, a chondrodystrophy, the osteoarthritis, a deformans discopathy, restoration / healing incompetence of fracture, etc. are illustrated. With the advent of the occupational disease especially represented by the increase in the lesion by arrival of an aging society, and the sport, the keypuncher disease, etc., joint obstacle patients are increasing in number remarkably, and progress of the medicine in this field is demanded. How to suppress obstacles, such as an ache based on the disorder, by their not aiming direct at the dissolution of a cause and prescribing an anti-inflammatory agent etc. for the patient, although various cures have been tried in order to treat cartilage disorder from the former for example; they could not but be symptomatic therapy-things, such as the method of injecting a hyaluronic-acid tablet etc. into a joint and making movement of a joint lubricous. Thus, the radical cure-cure for a joint obstacle is not found out, but especially the osteoarthritis has many patients and it is anxious for the effective cure.
[0004] In order to solve the above-mentioned technical problem, as a result of inquiring wholeheartedly, this invention persons have the operation which HGF promotes proliferation of a chondrocyte and promotes generation of a proteoglycan, found out that it was effective in the treatment of the various disorders resulting from cartilage disorder, and completed this invention. Above HGF is a hepatocyte. in vitro As a factor to proliferate It is found-out protein (). [Biochem] Biophys Res Commun, 122, 1450, 1984, and Proc. Natl.Acad.Sci.USA, 83, 6489, 1986, FFBS Letter, 22, 311, 1987, Nature, 342, 440, 1989, Proc.Natl.Acad.Sci.USA, 87, 3200, 1990. It became clear that various activity, such as organization trauma healing, is shown in the living body by the latest research result by many researchers including this invention persons, and expectation has gathered for the application to Homo sapiens, the therapeutic drug of an animal, etc. only as a candidate for research. It is shown clearly that the so-called paracrine mechanism in which it is solved that HGF is mainly produced by the cell of a mesenchyme system, and HGF is supplied if needed from a neighboring cell is materialized. However, since production of HGF increases also in the internal organs which have not

received the trauma, for example, lungs etc., when a trauma is received in liver or a kidney, it is thought that HGF is supplied by the so-called endocrine mechanism. It became deterministic about such an acceptor of HGF that the c-Met field oncogene is carrying out the code of the HGF acceptor from the latest research (Bottaro et al., Science 251, 802-804, 1991; Naldini et al., Oncogene 6, 501-504, 1991). Although many knowledge is acquired about HGF as mentioned above, a chondrocyte proliferation promotion operation of HGF and a proteoglycan generation promotion operation are new knowledge which is not known conventionally, and this invention made based on this knowledge is to provide the treatment of the various disorders resulting from cartilage disorder with a useful medicine.

[0005]

[Means for Solving the Problem] this invention made in order to solve the above-mentioned technical problem is a cartilage-disorder treatment agent characterized by containing **HGF as an active principle.;

** Chondrocyte proliferation accelerator characterized by containing HGF as an active principle;

** It is related with the proteoglycan generation accelerator characterized by containing HGF as an active principle.

[0006] If refined as HGF used by this invention by the grade which can be used as physic, what was prepared by various methods can be used. Various kinds of methods are learned as the manufacture method of HGF. For example, it can extract and refine and can obtain from blood cells, such as internal organs, such as the liver of mammalians, such as a rat, a cow, a horse, and a sheep, a spleen, a lung, bone marrow, a brain, a kidney, and a placenta, a platelet, and a leucocyte, plasma, a blood serum, etc. (references, such as FEBS Letters, 224, 312, 1987, Proc.Natl.Acad.Sci.USA, 86, 5844, and 1989). Moreover, the primary-culture cell and established cell line which produce HGF can be cultivated, separation refining can be carried out from a culture (a culture supernatant, cultured cell, etc.), and HGF can also be obtained. Or the recombination HGF which includes the gene which carries out the code of the HGF by the genetic engineering-technique in a suitable vector, inserts and carries out the transformation of this to a suitable host, and is made into the purpose from the culture supernatant of this transformant can be obtained (for example, references, such as Nature, 342, 440, 1989, JP,5-111383,A, Biochem.Biophys.Res.Commun., 163, 967, and 1989). Especially the above-mentioned host cell is not limited, but can use various kinds of host cells used by the genetic engineering-technique from the former, for example, Escherichia coli, a Bacillus subtilis, yeast, mold, vegetation, or an animal cell.

[0007] More specifically, as a method of carrying out extraction refining of the HGF from a body tissue, a carbon tetrachloride can be injected intraperitoneally to a rat, the liver of the rat changed into the hepatitis state can be extracted and ground, and it can refine in the usual protein purification methods, such as gel column chromatographies, such as S-sepharose and heparin sepharose, and HPLC, for example. Moreover, using the recombining-gene method, by the expression vector which included the gene which carries out the code of Homo sapiens's HGF amino acid sequence in vectors, such as a bovine papilloma virus DNA, the transformation of an animal cell, for example, a Chinese hamster ovary cell (CHO) cell, mouse C127 cell, the ape COS cell, etc. can be carried out, and it can obtain from the culture supernatant.

[0008] as long as HGF obtained in this way is this effect as substantially as HGF -- that, and a part of other amino acid sequences are inserted, or 1 or two or more amino acid have combined with the amino terminus and/or the C terminus **** -- or a sugar chain -- the same -- a deletion -- or it may be replaced [that a part of the amino acid sequence is replaced by a deletion or other amino acid]

[0009] As the treatment agent and accelerator of this invention make above HGF an active principle and HGF is shown in the example of the after-mentioned examination, it has the operation which promotes proliferation of a chondrocyte and promotes generation of a proteoglycan. Furthermore, HGF does not show an operation to the cartilaginous tissue which has not received the obstacle, but since it acts only on the cartilaginous tissue which has received the obstacle, it has the feature that few possibilities of causing a side effect are. Therefore, the treatment agent and accelerator of this invention are effective in the treatment and prevention of the various disorders resulting from cartilage disorder, and the following disorder is included by these.

[0010] Restoration by the articular cartilage by healing and the restoration lesion of hypertrophic-arthritis chondrodystrophy fracture, the restoration acute suppurative-arthritis tuberculous-arthritis arthritis-syphilitica rheumatoid arthritis rheumatic fever of the discus articularis radioulnaris, and the systemic-lupus-erythematosus spondylosis-deformans herniated disk bone grafting [0011] The treatment agent and accelerator of this invention are applied to the treatment and prevention of the various disorders resulting from the cartilage disorder in mammalians (for example, a cow, a horse, a swine, a sheep, a dog, a cat, etc.) besides Homo sapiens.

[0012] Although the treatment agent and accelerator of this invention can take various formulation (for example, solution, a solid preparation, a capsule, etc.), let them be the injection, the vapor, a suppository, or an oral agent with the support of it and common use of only HGF which is generally an active principle. The injection concerned can be prepared by the conventional method, for example, HGF can be filtered with a filter etc., after dissolving in suitable

solvents (for example, the sterilized water, the buffer solution, a physiological saline, etc.), and it can sterilize, and it can be prepared by filling up a sterile container subsequently. As a HGF content in the injection, it is usually preferably adjusted to 0.001 to 0.1 (W/V %) grade 0.0002 to 0.2 (W/V %) grade. Moreover, as an oral medicine, it is tablet-ized by dosage forms, such as a tablet, a granule, a fine-grain agent, powder, ** or hard capsules, solution, an emulsion, suspension, and syrup, and these tablets can be prepared according to the conventional method of tablet-izing, for example. A suppository can also be prepared by the conventional method on the tablets (for example, cacao butter, Rau Lynne fat, a glycerogelatin, macro gall, witepsol, etc.) using the basis of common use. Moreover, the vapor can also be prepared according to the stock-in-trade on a tablet. The HGF content in a tablet can be suitably adjusted according to dosage forms, an application disorder, etc.

[0013] On the occasion of tablet-izing, a stabilizing agent is added preferably and albumin, a globulin, gelatin, a glycine, a mannitol, a glucose, a dextran, a sorbitol, ethylene glycol, etc. are mentioned as a stabilizing agent, for example. Furthermore, the tablet of this invention may contain an additive required for tablet-izing, for example, an excipient, the solubilizing agent, the antioxidant, the analgesia-ized agent, the isotonizing agent, etc. When it considers as liquid preparations, it is desirable for cryopreservation or freeze drying to remove moisture and to save. lyophilized products -- business -- it is used for it, sometimes adding the water for injection etc. and sometimes remelting

[0014] The treatment agent and accelerator of this invention may be prescribed for the patient by the suitable route of administration according to the formulation. For example, it can be made the gestalt of the injection and a medicine can be prescribed for the patient into a vein, an artery, hypodermically, and muscles etc. Although the dose is suitably adjusted by a patient's symptom, age, weight, etc., usually, as HGF, it is 1mg - 100mg preferably, and it is appropriate for it to prescribe [0.05mg - 500mg] this for the patient in 1 time per or several steps day.

[0015]

[Effect of the Invention] In this invention, HGF which is an active principle has the operation which proliferation of a chondrocyte is promoted [operation] and promotes generation of a PURITEO glycan. Therefore, the treatment agent and accelerator of this invention are useful to the treatment and prevention of the various disorders resulting from the cartilage disorder mentioned above. Furthermore, since HGF acts only on the cartilaginous tissue which has received the obstacle, it does so the effect that a medicine with few side effects can be obtained.

[0016]

[Example] Hereafter, although this invention is explained more to a detail based on an example and the example of manufacture, this invention is not limited to these examples. In addition, the material and the method which were used in the following experiments are as follows.

[0017] 1.4kb(s) of material and method **in situ hybridization rat HGF-cDNA (red-blood-cell1 clone) (Proc.Natl.Acad.Sci.USA, 87, 3200, 1990) The RNA probe of an anti sense and a sense which carried out the sub clone of the EcoRI fragment to pGEM7 vector, and labeled by [a-35S] UTP (400 Ci/mmol and Amersham) was produced. The imprint object which carried out the indicator carried out alkali hydrolysis as a RIBOPU lobe at 50 -150 base. in situ hybridization was carried out by the method given in reference (Biochem.Biophys.Res.Commun., 173, 42, 1990). The sample was fixed with the 4% paraformaldehyde-phosphoric-acid physiological saline solution, and it was made dehydration by ethanol and it made embedding to paraffin after washing with toluene. The 5-micrometer intercept was cut down and it mounted on the slide glass which carried out the coat by poly-L-lysine. Deparaffinization of the intercept was carried out by the glycine and the acetic anhydride, and it was hybridized with 50 degrees C and the 16-hour probe. Then, after 0.1xSSC liquid washed 50 degrees C of intercepts for 1 hour and processing them 37 degrees C by RNAaseA (20microg/(ml)) for 30 minutes, 2xSSC liquid washed twice in 37 degrees C and 10 minutes. The intercept was dipped in the emulsion (1:1 KODAKKU NBT-2 diluent), and was exposed for two weeks. The intercept carried out development fixing and was dyed KODAKKU D-19 by hematoxylin and eosin.

[0018] ** The cell and the cell culture chondrocyte were isolated from 23 age-in-day embryos and the 4-weeks old newborn infant of the New Zealand white rabbit according to the method given in reference (J.Cell.Physiol., 133, 491, 1987). The articular cartilage isolated a femur articular cartilage in the knees to the costal cartilage from the hyaline cartilage of a rib (Dev.Biol., 136, 500, 1989). The synovial membrane fibroblast was isolated from the synovial membrane organization of a knee joint. The cells which cultivated the synovial membrane organization fragment which carried out the fragment for ten days by DMEM which contains FBS 10%, and were increased by trypsinization were collected. According to the method given in reference (Exp.Cell Res., 157, 483, 1985), the viviparous mesenchyme system cell was isolated from 20 age-in-day rat embryo's foot muscular system. The **** mesenchyme cell was isolated from the rat embryo of 10.5 ages in day. **** was started under the surgical microscope, and it 0.25% with the trypsin, and it obtained the isolation cell with nylon gauze. [in 30 minutes] [after processing] All the cells were maintained 10% under 37 degrees C and 5% 2/95% air of COs by FBS and DMEM (henceforth a culture medium A) containing a

60microg [/ml] kanamycin except the foot blast cell.
[0019] ** Measurement of the incorporation of the [3H]-thymidine ([6-3H]-thymidine, Amersham, 20 Ci/mmol) to 10% TCA insoluble sexual-cell precipitation estimated the measurement DNA synthesis speed of DNA synthesis (J.Clin.Invest., 85, 626, 1990). a cell -- 96 hole plate -- it cultivated until it carried out seeding and became confluent by the density of 1.5x104 per well 6mm In order to stop proliferation, the pre incubation of the cell was carried out by 0.1ml of the FBS content DMEM 0.3%. HGF of various concentration was added to the culture medium. The incubation was continued for 24 hours. 1microcurie [/ml] [3-H] thymidine was added 3 hours before the incubation halt. The ethanol:wood ether (3:1) washed the after [an indicator] cell once twice by 5%TCA which contains 3mM thymidine 3 times by Ice-cooling PBS. a well -- the inner residue was solubilized by 0.1N NaOH of 100microl, was moved to the liquid SHINCHI vial, and measured radioactivity with the scintillation counter (Rack-beta, Pharmacia Corp.) after neutralization by 1N HCl
[0020] ** Seeding of the measurement chondrocyte of proteoglycan composition was carried out by the density of 1.5x104 per well 6mm, and it was maintained by the 0.1ml culture medium A. When the cell reached confluent, the pre incubation was carried out by 0.1ml DMEM which contains FBS 0.3% for 24 hours. Then, the incubation was carried out by 0.1ml DMEM which contains FBS and HGF 0.3% for 24 hours. The 1microcurie [/ml] [35S]-sulfuric-acid machine was added 20 hours before the incubation end. Measurement of the incorporation of the [35S]-sulfuric-acid machine to the precipitate in the cetyl pyridinium chloride after protease digestion estimated proteoglycan composition (Exp.Cell Res., 130, 73, 1980).
[0021] ** The total RNA was prepared from the total RNA manufacture and the reverse transcription PCR cartilage by the strange method of a method given in reference (Anal.Biochem., 203, 352, 1992). A fresh isolation organization fragment (0.1g wet weight) is 4M guanidine thiocyanate and 0.1M. The homogenate was quickly carried out by 2ml of 4M GITC solutions of a Tris hydrochloric acid (pH 7.5) and a 1%2-mercaptoethanol. It mixed to 10%SDS100microl and the at-long-intervals heart of the homogenate was carried out with the minute amount centrifuge for 5 minutes. They are 1.6g caesium trifluoroacetate of this capacity, and 1mM in the Beckmann polyallomer centrifugal tube (13x51mm) in 2ml of supernatant liquids. It carried out multistory to EDTA (pH 8.0). It carried out centrifugal [of the 18 degrees C of the samples] by 35,000rpm (147,000xg) for 20 hours. The supernatant liquid was dissolved after suction removal, precipitation was dissolved in 200micro of 4M GITC solutions l, 3M sodium acetate (pH 4.8) of 20microl was mixed after extraction processing by phenol:chloroform:isoamyl alcohol (25:24:1), and it was made to precipitate by the ethanol of double-precision capacity (440microl). Dregs were dissolved in the DEPC treated water. First, composition of the first strand cDNA was performed using the anti sense primer of a SuperScript inversion enzyme (Gibco-BRL) and a down-stream region from the 0.5microg total RNA. PCR proliferation was performed succeedingly. 58 degrees C was performed proliferation for 30 seconds at 94 degrees C, and it was performed at 72 degrees C for 1 minute on condition that 35 cycles (in the case of a chondrocyte) in 1.5 minutes, or 40 cycles (in the case of a cartilaginous tissue). The primer base sequence of PCR proliferation produced the 725bp fragment by 5 '-CAGT(A/G) ATGATCTCAATGGGCAAT-3' and 5'-AATGCCCTCTTCCTATGACTTC-3' to c-Met (Oncogene, 2, 593, 1988) of a rat and a mouse.
[0022] The manifestation of HGFmRNA in the limb bud of the HGFmRNA manifestation nascent state mouse in the example 1 generating member was examined by the in situ hybridization method. The result is shown in drawing 1 , drawing 2 , and drawing 3 . Drawing 1 shows the manifestation of HGFmRNA in the limb bud of an early nascent state mouse, it is the microphotography of the longitudinal-section intercept of a hind foot, and light field (left-hand side) and the dark field (right-hand side) corresponding to it are photoed after in situ hybridization, an autoradiography, and dyeing. In this drawing, A-D is 10.5 age-in-day embryos, and E-H is 11 age-in-day embryos' intercept. Drawing 2 shows the manifestation of HGFmRNA in the limb bud of a finger formative period mouse, it is the microphotography of the longitudinal-section intercept of a hind foot, and light field (left-hand side) and the dark field (right-hand side) corresponding to it are photoed after in situ hybridization, an autoradiography, and dyeing. In this drawing, A and B are [13 age-in-day embryos and G-J of 12.5 age-in-day embryos and C-F] 14 age-in-day embryos' intercepts. Moreover, in a femur and Fi, a fibula and Ta show the tarsitis and I-V shows [Fe] a finger number. Drawing 3 is a microphotography in which the manifestation of HGFmRNA in the limb bud and thorax of a nascent state mouse is shown, and light field (left-hand side) and the dark field (right-hand side) corresponding to it are photoed after in situ hybridization, an autoradiography, and dyeing. this drawing -- setting -- A and B -- the cross-section intercept of 16 age-in-day embryos' hind foot --; -- C and D show 13 age-in-day embryos, and E and F show the longitudinal-section intercept of 14 age-in-day embryos' thorax Moreover, in Ta, the tarsitis and Ti show a tibia and Rib shows precartilage nature accumulation of a rib cartilage.
[0023] As shown in drawing 1 , the diffusion manifestation of HGFmRNA was detected around [a pars-basilaris-ossis-

occipitalis field] the member on the 11th. Cartilaginous accumulation was not generated in the member in this stage. Advance of cartilaginous accumulation has restricted the manifestation part of HGFmRNA more. When the stylopodium, a junction foot, and an autopodium portion were formed on the 12.5th, the manifestation of HGFmRNA was observed in the joint field of a wrist/malleolus, and an elbow/knee (refer to drawing 2 A and B.). For convenience, the knee and the malleolus were shown. HGFmRNA was discovered in the second half (13 - 14 days) into the mesenchyme system cell which cartilage accumulation of the joint field of a wrist/malleolus, and an elbow/knee adjoined, and was limited (refer to drawing 2 C-J). HGFmRNA was localized to the limited mesenchyme cell which adjoins the cartilage of the tarsitis on the 16th (refer to drawing 3 A and B). The manifestation level in the member of HGFmRNA decreased with specialization. The growth board of hand and foot did not detect HGFmRNA through the examination.

[0024] The manifestation of HGFmRNA in the thorax of the HGFmRNA manifestation nascent state mouse in the example 2 generating thorax was examined by the in situ hybridization method. The result is shown in drawing 3 C-F. As shown in drawing 3 C-F, HGFmRNA was discovered by the circumference interspace mesenchyme at the nose of cam the precartilage nature accumulation which the interspace elongated. The signal of hybridization was not detected in precartilage nature accumulation.

[0025] In order that which might decide whether to be the target cell of HGF around the cartilaginous tissue to the chondrocyte of example 3HGF which is carrying out SUKYATTA activity test specialization, from the chondrocyte from a knee-joint cartilage and a costal cartilage, and the knee joint, the cultured cell of the synovial cell and the fibroblast increased from the member muscular system was prepared, and the effect of HGF added in external cause into these cells was examined. That is, seeding of the rabbit articular cartilage cell was carried out to the well by the density of 3x103 cells 16mm, and it maintained for two days by the culture medium A. Then, processing was performed for two days by HGF. The phase contrast microphotography was taken at the time of the end of an incubation. The result is shown in drawing 4 . As shown in drawing 4 , in HGF un-processing (control), the polygonal chondrocyte increased and it became island-like (drawing 4 A). On the other hand, in the cultivation which processed by HGF (3 ng/ml), the chondrocyte did not form an island in the state of the single cell (drawing 4 B). Therefore, it became clear that HGF stimulates movement of a chondrocyte. In addition, HGF was not distributed about the fibroblast and the synovial cell.

[0026] The effect of HGF to proliferation of the effect chondrocyte of HGF to example 4 chondrocyte proliferation was examined. That is, the articular cartilage cell extracted from the 4-weeks old rabbit was cultivated. The amount of incorporation of [3H]-thymidine was measured by the method which dealt with the cell which became confluent by HGF of various concentration, and showed it to the term of material and a method after carrying out blood serum removal processing for 24 hours. Moreover, the examination with the same said of the rabbit synovial membrane fibroblast was performed. The result is shown in drawing 5 A (articular cartilage cell) and B (synovial membrane fibroblast). In addition, a result shows the average ** standard deviation of three examinations (also setting to drawing 5 C, drawing 6 , and drawing 7 A and B the same). As shown in drawing 5 A, HGF made the incorporation of the [3H]-thymidine to a rabbit articular cartilage cell increase in dosage dependence, and having promotion of DNA synthesis, i.e., the proliferation promotion operation to an articular cartilage cell, was shown. In 1 ng/ml HGF, as for DNA synthesis, the 3 times as many increase as this was accepted to control. On the other hand, as shown in drawing 5 B, the synovial membrane fibroblast did not react to HGF.

[0027] Moreover, another experiment examined the effect of HGF to the number of cells of an articular cartilage. That is, 1x104 cell seeding of the rabbit articular cartilage cell was carried out to the well 16mm, and it maintained by the DMEM culture medium which contains FBS 10%. Subsequently, the incubation of the 10 ng/ml HGF was added and carried out for 48 hours, and the number of cells was measured after the incubation end. The result is shown in drawing 5 C. 10 ng/ml HGF made the number of cells increase by about 1.8 times compared with control, as shown in drawing 5 C.

[0028] the effect of HGF to example 5 proteoglycan generation -- as mentioned above, since HGF promoted multiplication of a chondrocyte next, the effect over proteoglycan generation of an articular cartilage cell was examined by the method shown by the above-mentioned material and the term of a method Measurement of incorporation of the [35S]-sulfuric-acid machine to the macromolecule (glycosaminoglycan) which precipitates by the cetyl pyridinium chloride after protease digestion considered proteoglycan composition (Exp.Cell Res., 130, 73, 1980). In addition, it replaced with HGF and examined also about the following factor.

insulin Mr. growth factor (IGF)-I:concentration 100 ng/mlIGF-II:100 ng/ml concentration parathyroid-hormone (PTH): -- concentration 10-7 MTGF-beta: -- the result is shown in drawing 6 3 ng/ml concentration HGF made the incorporation of a [35S]-sulfuric-acid machine increase in dosage dependence, as shown in drawing 6 . The maximum increase was acquired by 1 ng/ml HGF. Although this operation was weaker than TGF-beta (J.Cell Physiol., 138, 329, 1989) and PTH (J.Clin.Invest., 85, 626, 1990), IGF-I and its II were of the same grade (Exp.Cell Res., 130, 73, 1980).

[Q029] the effect of HGF to the DNA synthesis and proteoglycan generation under example 6 anti-HGF antibody existence -- it is thought that HGF generally acts on a target cell by the paracrine mechanism as mentioned above, and it is considered that the result of the aforementioned in situ hybridization is supporting this idea Then, in order to check this point, the HGF polyclonal antibody examined whether the function of a chondrocyte would be changed. namely, the rabbit articular cartilage cell which became confluent -- the bottom of existence of 3 ng/ml HGF or nonexistence, and an anti-[25microg //ml] HGF polyclonal antibody (IgG fraction refined by the affinity) -- processing -- or it un-processed Then, by the method shown by the aforementioned material and the term of a method, the indicator was carried out with [3H]-thymidine or the [35S]-sulfuric-acid machine, and DNA synthesis or proteoglycan generation was measured. The result is shown in drawing 7 A (DNA synthesis) and B (proteoglycan generation). In addition, in this drawing, Ab shows an anti-HGF polyclonal antibody. As shown in drawing 7 , neither the DNA synthesis in an articular cartilage cell nor proteoglycan generation was changed only by addition of an anti-HGF polyclonal antibody. However, the anti-HGF polyclonal antibody checked completely the effect of HGF added to exogenism. This shows that sufficient HGF for a chondrocyte to adjust the own function of a cartilage is not produced.
[0030] Reverse transcription PCR considered the manifestation of the HGF receptor (c-Met) in the chondrocyte besides [manifestation in-the-living-body / in the chondrocyte of the example 7HGF receptor mRNA / and] a living body. Each portion of a joint organization and a costal cartilage was started from the 4-weeks old rat under the surgical microscope, and the total RNA was extracted as the term of a method and material described. After carrying out reverse transcription of the extracted RNA (0.5microg) and amplifying it using the primer of c-Met, it was analyzed by agarose gel electrophoresis 1.5%. The result is shown in drawing 8 . As shown in drawing 8 , the c-Met manifestation of a minute amount was detected after 40 amplification about the articular cartilage organization and the costal cartilage organization, and the cultivation chondrocyte accepted the Tsuguaki c-Met manifestation after 35 amplification.
[0031] Example of production of an example of manufacture 1HGF tablet (1) HGF 20microg human serum albumin The 100mg above-mentioned matter was dissolved by PBS of 0.01M of pH 7.0, the whole quantity was prepared to 20ml, 2ml was poured distributively into each vial bottle after sterilization, and freeze-drying seal was carried out.
(2) HGF 40microg Tween 80 1mg human serum albumin The 100mg above-mentioned matter was dissolved in the physiological saline for injection, the whole quantity was prepared to 20ml, 2ml was poured distributively into each vial bottle after sterilization, and freeze-drying seal was carried out.

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TECHNICAL FIELD

[Industrial Application] this invention relates to the cartilage-disorder treatment agent, chondrocyte proliferation accelerator, and proteoglycan generation accelerator which contain HGF (Hepatocyte Growth Factor) as an active principle in a detail more about a medicine useful to the treatment and prevention of a chondropathy.

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PRIOR ART

[Description of the Prior Art] A cartilage is a connective tissue which consists of a substrate which encloses a chondrocyte and this, and exists in a joint, the intervertebral disk of a spine, a costal cartilage, a pinna, external auditory meatus, pubic symphysis, a throat lid, etc. A cartilage consists of cartilage matrix which a chondrocyte and a chondrocyte produce, cartilage matrix is the components with main fiber component, proteoglycan, and water, such as a collagen fiber, and a cartilage can be classified into hyaline cartilage (a costal cartilage, a throat cartilage, articular cartilage, etc.), elastic cartilages (conchal cartilage etc.), and fibrocartilage (an intervertebral disk cartilage, a pubis cartilage, articular cartilage, etc.) according to mixture ****** of the cartilage matrix. In the above-mentioned cartilage matrix, a collagen fiber participates in the rigidity and intensity to the tension and shearing force of a cartilage, a proteoglycan participates in the intensity to compressive force, it is supposed that water is kept in the property as a viscoelastic body as a body tissue, for example, in the case of the articular cartilage, water occupied 78.6% of the nature weight of a cartilage, the collagen occupied 20%, and the proteoglycan occupies 7%. As an operation of a cartilage, reduction (cartilage between bones) of friction of epiphysis, maintenance (conchal cartilage etc.) of elasticity, and motor functions (a costal cartilage, pubis cartilage, etc.) are mentioned.

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EFFECT OF THE INVENTION

[Effect of the Invention] In this invention, HGF which is an active principle has the operation which multiplication of a chondrocyte is promoted [operation] and promotes generation of a PURITEO glycan. Therefore, the medical treatment agent and accelerator of this invention are useful to the medical treatment and prevention of the various diseases resulting from the cartilage obstacle mentioned above. Furthermore, since HGF acts only on the cartilaginous tissue which has received the obstacle, it does so the effect that a medicine with few side effects can be obtained.

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TECHNICAL PROBLEM

[Problem(s) to be Solved by the Invention] As mentioned above, the cartilage has the important operation, after that a living body maintains [functional], the various disorders which originate in the obstacle of a cartilage from the former are known, for example, a chondrodystrophy, the osteoarthritis, a deformans discopathy, restoration / healing incompetence of fracture, etc. are illustrated. With the advent of the occupational disease especially represented by the increase in the lesion by arrival of an aging society, and the sport, the keypuncher disease, etc., joint obstacle patients are increasing in number remarkably, and progress of the medicine in this field is demanded. How to suppress obstacles, such as an ache based on the disorder, by their not aiming direct at the dissolution of a cause and prescribing an anti-inflammatory agent etc. for the patient, although various cures have been tried in order to treat cartilage disorder from the former for example; they could not but be symptomatic therapy-things, such as the method of injecting a hyaluronic-acid tablet etc. into a joint and making movement of a joint lubricous. Thus, the radical cure-cure for a joint obstacle is not found out, but especially the osteoarthritis has many patients and it is anxious for the effective cure.
[0004] In order to solve the above-mentioned technical problem, as a result of inquiring wholeheartedly, this invention persons have the operation which HGF promotes proliferation of a chondrocyte and promotes generation of a proteoglycan, found out that it was effective in the treatment of the various disorders resulting from cartilage disorder, and completed this invention. Above HGF is a hepatocyte. in vitro As a factor to proliferate It is found-out protein (). [Biochem] Biophys Res Commun, 122, 1450, 1984, and Proc. Natl.Acad.Sci.USA, 83, 6489, 1986, FFBS Letter, 22, 311, 1987, Nature, 342, 440, 1989, Proc.Natl.Acad.Sci.USA, 87, 3200, 1990. It became clear that various activity, such as organization trauma healing, is shown in the living body by the latest research result by many researchers including this invention persons, and expectation has gathered for the application to Homo sapiens, the therapeutic drug of an animal, etc. only as a candidate for research. It is shown clearly that the so-called paracrine mechanism in which it is solved that HGF is mainly produced by the cell of a mesenchyme system, and HGF is supplied if needed from a neighboring cell is materialized. However, since production of HGF increases also in the internal organs which have not received the trauma, for example, lungs etc., when a trauma is received in liver or a kidney, it is thought that HGF is supplied by the so-called endocrine mechanism. It became deterministic about such an acceptor of HGF that the c-Met field oncogene is carrying out the code of the HGF acceptor from the latest research (Bottaro et al., Science 251, 802-804, 1991; Naldini et al., Oncogene 6, 501-504, 1991). Although many knowledge is acquired about HGF as mentioned above, a chondrocyte proliferation promotion operation of HGF and a proteoglycan generation promotion operation are new knowledge which is not known conventionally, and this invention made based on this knowledge is to provide the treatment of the various disorders resulting from cartilage disorder with a useful medicine.

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MEANS

[Means for Solving the Problem] this invention made in order to solve the above-mentioned technical problem is cartilage obstacle medical treatment agent; characterized by containing **HGF as an active principle.
** Chondrocyte multiplication accelerator; characterized by containing HGF as an active principle.
** It is related with the proteoglycan generation accelerator characterized by containing HGF as an active principle.
[0006] If refined as HGF used by this invention by the grade which can be used as medicine, what was prepared by various methods can be used. Various kinds of methods are learned as the manufacture method of HGF. For example, it can extract and refine and can obtain from blood cells, such as internal organs, such as the liver of mammalians, such as a rat, a cow, a horse, and a sheep, a spleen, a lung, bone marrow, a brain, the kidney, and a placenta, a platelet, and a leucocyte, plasma, a blood serum, etc. (references, such as FEBS Letters, 224, 312, 1987, Proc.Natl.Acad.Sci.USA, 86, 5844, and 1989). Moreover, the primary-culture cell and established cell line which produce HGF can be cultivated, separation refining can be carried out from a culture (a culture supernatant, cultured cell, etc.), and HGF can also be obtained. Or the recombination HGF which includes the gene which carries out the code of the HGF by the genetic engineering-technique in a suitable vector, inserts and carries out the transformation of this to a suitable host, and is made into the purpose from the culture supernatant of this transformant can be obtained (for example, references, such as Nature, 342, 440, 1989, JP,5-111383,A, Biochem.Biophys.Res.Commun., 163, 967, and 1989). Especially the above-mentioned host cell is not limited, but can use various kinds of host cells used by the genetic engineering-technique from the former, for example, Escherichia coli, a Bacillus subtilis, yeast, mold, vegetation, or an animal cell.
[0007] More specifically, as a method of carrying out extraction refining of the HGF from a body tissue, a carbon tetrachloride can be injected intraperitoneally to a rat, the liver of the rat changed into the hepatitis state can be extracted and ground, and it can refine in the usual protein purification methods, such as gel column chromatographies, such as S-sepharose and heparin sepharose, and HPLC, for example. Moreover, using the recombining-gene method, by the expression vector which included the gene which carries out the code of Homo sapiens's HGF amino acid sequence in vectors, such as a bovine papilloma virus DNA, the transformation of an animal cell, for example, a Chinese hamster ovary cell (CHO) cell, mouse C127 cell, the ape COS cell, etc. can be carried out, and it can obtain from the culture supernatant.
[0008] as long as HGF obtained in this way is this effect as substantially as HGF -- that, and a part of other amino acid sequences are inserted, or 1 or two or more amino acid have combined with the amino terminus and/or the C terminus **** -- or a sugar chain -- the same -- a deletion -- or it may be replaced [that a part of the amino acid sequence is replaced by a deletion or other amino acid]
[0009] As the treatment agent and accelerator of this invention make above HGF an active principle and HGF is shown in the example of the after-mentioned examination, it has the operation which promotes proliferation of a chondrocyte and promotes generation of a proteoglycan. Furthermore, HGF does not show an operation to the cartilaginous tissue which has not received the obstacle, but since it acts only on the cartilaginous tissue which has received the obstacle, it has the feature that few possibilities of causing a side effect are. Therefore, the treatment agent and accelerator of this invention are effective in the treatment and prevention of the various disorders resulting from cartilage disorder, and the following disorder is included by these.
[0010] Restoration by the articular cartilage by healing and the restoration lesion of hypertrophic-arthritis chondrodystrophy fracture, the restoration acute suppurative-arthritis tuberculous-arthritis arthritis-syphilitica rheumatoid arthritis rheumatic fever of the discus articularis radioulnaris, and the systemic-lupus-erythematosus spondylosis-deformans herniated disk bone grafting. [0011] The treatment agent and accelerator of this invention are applied to the treatment and prevention of the various disorders resulting from the cartilage disorder in mammalians (for example, a cow, a horse, a swine, a sheep, a dog, a cat, etc.) besides Homo sapiens.

[Q012] Although the treatment agent and accelerator of this invention can take various formulation (for example, solution, a solid preparation, a capsule, etc.), let them be the injection, the vapor, a suppository, or an oral agent with the support of it and common use of only HGF which is generally an active principle. The injection concerned can be prepared by the conventional method, for example, HGF can be filtered with a filter etc., after dissolving in suitable solvents (for example, the sterilized water, the buffer solution, a physiological saline, etc.), and it can sterilize, and it can be prepared by filling up a sterile container subsequently. As a HGF content in the injection, it is usually preferably adjusted to 0.001 to 0.1 (W/V %) grade 0.0002 to 0.2 (W/V %) grade. Moreover, as an oral medicine, it is tablet-ized by dosage forms, such as a tablet, a granule, a fine-grain agent, powder, ** or hard capsules, solution, an emulsion, suspension, and syrup, and these tablets can be prepared according to the conventional method of tablet-izing, for example. A suppository can also be prepared by the conventional method on the tablets (for example, cacao butter, Rau Lynne fat, a glycerogelatin, macro gall, witepsol, etc.) using the basis of common use. Moreover, the vapor can also be prepared according to the stock-in-trade on a tablet. The HGF content in a tablet can be suitably adjusted according to dosage forms, an application disorder, etc.

[0013] On the occasion of tablet-izing, a stabilizing agent is added preferably and albumin, a globulin, gelatin, a glycine, a mannitol, a glucose, a dextran, a sorbitol, ethylene glycol, etc. are mentioned as a stabilizing agent, for example. Furthermore, the tablet of this invention may contain an additive required for tablet-izing, for example, an excipient, the solubilizing agent, the antioxidant, the analgesia-ized agent, the isotonizing agent, etc. When it considers as liquid preparations, it is desirable for cryopreservation or freeze drying to remove moisture and to save. lyophilized products -- business -- it is used for it, sometimes adding the water for injection etc. and sometimes remelting

[0014] The treatment agent and accelerator of this invention may be prescribed for the patient by the suitable route of administration according to the formulation. For example, it can be made the gestalt of the injection and a medicine can be prescribed for the patient into a vein, an artery, hypodermically, and muscles etc. Although the dose is suitably adjusted by a patient's symptom, age, weight, etc., usually, as HGF, it is 1mg - 100mg preferably, and it is appropriate for it to prescribe [0.05mg - 500mg] this for the patient in 1 time per or several steps day.

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EXAMPLE

[Example] Hereafter, although this invention is explained more to a detail based on an example and the example of manufacture, this invention is not limited to these examples. In addition, the material and the method which were used in the following experiments are as follows.
[0017] 1.4kb(s) of material and method **in situ hybridization rat HGF-cDNA (red-blood-cell1 clone) (Proc.Natl.Acad.Sci.USA, 87, 3200, 1990) The RNA probe of an anti sense and a sense which carried out the sub clone of the EcoRI fragment to pGEM7 vector, and labeled by [a-35S] UTP (400 Ci/mmol and Amersham) was produced. The imprint object which carried out the indicator carried out alkali hydrolysis as a RIBOPU lobe at 50 -150 base. in situ hybridization was carried out by the method given in reference (Biochem.Biophys.Res.Commun., 173, 42, 1990). The sample was fixed with the 4% paraformaldehyde-phosphoric-acid physiological saline solution, and it was made dehydration by ethanol and it made embedding to paraffin after washing with toluene. The 5-micrometer intercept was cut down and it mounted on the slide glass which carried out the coat by poly-L-lysine. Deparaffinization of the intercept was carried out by the glycine and the acetic anhydride, and it was hybridized with 50 degrees C and the 16-hour probe. Then, after 0.1xSSC liquid washed 50 degrees C of intercepts for 1 hour and processing them 37 degrees C by RNAaseA (20microg/(ml)) for 30 minutes, 2xSSC liquid washed twice in 37 degrees C and 10 minutes. The intercept was dipped in the emulsion (1:1 KODAKKU NBT-2 diluent), and was exposed for two weeks. The intercept carried out development fixing and was dyed KODAKKU D-19 by hematoxylin and eosin.
[0018] ** The cell and the cell culture chondrocyte were isolated from 23 age-in-day embryos and the 4-weeks old newborn infant of the New Zealand white rabbit according to the method given in reference (J.Cell.Physiol., 133, 491, 1987). The articular cartilage isolated a femur articular cartilage in the knees to the costal cartilage from the hyaline cartilage of a rib (Dev.Biol., 136, 500, 1989). The synovial membrane fibroblast was isolated from the synovial membrane organization of a knee joint. The cells which cultivated the synovial membrane organization fragment which carried out the fragment for ten days by DMEM which contains FBS 10%, and were increased by trypsinization were collected. According to the method given in reference (Exp.Cell Res., 157, 483, 1985), the viviparous mesenchyme system cell was isolated from 20 age-in-day rat embryo's foot muscular system. The **** mesenchyme cell was isolated from the rat embryo of 10.5 ages in day. **** was started under the surgical microscope, and it 0.25% with the trypsin, and it obtained the isolation cell with nylon gauze. [in 30 minutes] [after processing] All the cells were maintained 10% under 37 degrees C and 5% 2/95% air of COs by FBS and DMEM (henceforth a culture medium A) containing a 60microg [/ml] kanamycin except the foot blast cell.
[0019] ** Measurement of incorporation of the [3H]-thymidine ([6-3H]-thymidine, Amersham, 20 Ci/mmol) to 10% TCA insoluble sexual-cell precipitation estimated the measurement DNA synthesis speed of DNA synthesis (J.Clin.Invest., 85, 626, 1990). a cell -- 96 hole plate -- it cultivated until it carried out seeding and became confluent by the density of 1.5x104 per well 6mm In order to stop multiplication, the pre incubation of the cell was carried out by 0.1ml of the FBS content DMEM 0.3%. HGF of various concentration was added to the culture medium. The incubation was continued for 24 hours. 1microcurie [/ml] [3-H] thymidine was added 3 hours before the incubation halt. The ethanol:wood ether (3:1) washed the after [an indicator] cell once twice by 5%TCA which contains 3mM thymidine 3 times by Ice-cooling PBS. a well -- the inner residue was solubilized by 0.1N NaOH of 100microl, was moved to liquid SHINCHIBAIARU, and measured radioactivity with the scintillation counter (Rack-beta, Pharmacia Corp.) after neutralization by 1N HCl
[0020] ** Seeding of the measurement chondrocyte of proteoglycan composition was carried out by the density of 1.5x104 per well 6mm, and it was maintained by the 0.1ml culture medium A. When the cell reached confluent, the pre incubation was carried out by 0.1ml DMEM which contains FBS 0.3% for 24 hours. Then, the incubation was carried out by 0.1ml DMEM which contains FBS and HGF 0.3% for 24 hours. The 1microcurie [/ml] [35S]-sulfuric-acid

machine was added 20 hours before the incubation end. Measurement of the incorporation of the [35S]-sulfuric-acid machine to the precipitate in the cetyl pyridinium chloride after protease digestion estimated proteoglycan composition (Exp.Cell Res., 130, 73, 1980).

[0021] ** The total RNA was prepared from the total RNA manufacture and the reverse transcription PCR cartilage by the strange method of a method given in reference (Anal.Biochem., 203, 352, 1992). A fresh isolation organization fragment (0.1g wet weight) is 4M guanidine thiocyanate and 0.1M. The homogenate was quickly carried out by 2ml of 4M GITC solutions of a Tris hydrochloric acid (pH 7.5) and a 1%2-mercaptoethanol. It mixed to 10%SDS100microl and the at-long-intervals heart of the homogenate was carried out with the minute amount centrifuge for 5 minutes. They are 1.6g caesium trifluoroacetate of this capacity, and 1mM in the Beckmann polyallomer centrifugal tube (13x51mm) in 2ml of supernatant liquids. It carried out multistory to EDTA (pH 8.0). It carried out centrifugal [of the 18 degrees C of the samples] by 35,000rpm (147,000xg) for 20 hours. The supernatant liquid was dissolved after suction removal, precipitation was dissolved in 200micro of 4M GITC solutions l, 3M sodium acetate (pH 4.8) of 20microl was mixed after extraction processing by phenol:chloroform:isoamyl alcohol (25:24:1), and it was made to precipitate by the ethanol of double-precision capacity (440microl). Dregs were dissolved in the DEPC treated water. First, composition of the first strand cDNA was performed using the anti sense primer of a SuperScript inversion enzyme (Gibco-BRL) and a down-stream region from the 0.5microg total RNA. PCR proliferation was performed succeedingly. 58 degrees C was performed proliferation for 30 seconds at 94 degrees C, and it was performed at 72 degrees C for 1 minute on condition that 35 cycles (in the case of a chondrocyte) in 1.5 minutes, or 40 cycles (in the case of a cartilaginous tissue). The primer base sequence of PCR proliferation produced the 725bp fragment by 5 '-CAGT(A/G) ATGATCTCAATGGGCAAT-3' and 5'-AATGCCCTCTTCCTATGACTTC-3' to c-Met (Oncogene, 2, 593, 1988) of a rat and a mouse.

[0022] The manifestation of HGFmRNA in the limb bud of the HGFmRNA manifestation nascent state mouse in the example 1 generating member was examined by the in situ hybridization method. The result is shown in drawing 1 , drawing 2 , and drawing 3 . Drawing 1 shows the manifestation of HGFmRNA in the limb bud of an early nascent state mouse, it is the microphotography of the longitudinal-section intercept of a hind foot, and light field (left-hand side) and the dark field (right-hand side) corresponding to it are photoed after in situ hybridization, an autoradiography, and dyeing. In this drawing, A-D is 10.5 age-in-day embryos, and E-H is 11 age-in-day embryos' intercept. Drawing 2 shows the manifestation of HGFmRNA in the limb bud of a finger formative period mouse, it is the microphotography of the longitudinal-section intercept of a hind foot, and light field (left-hand side) and the dark field (right-hand side) corresponding to it are photoed after in situ hybridization, an autoradiography, and dyeing. In this drawing, A and B are [13 age-in-day embryos and G-J of 12.5 age-in-day embryos and C-F] 14 age-in-day embryos' intercepts. Moreover, in a femur and Fi, a fibula and Ta show the tarsitis and I-V shows [Fe] a finger number. Drawing 3 is a microphotography in which the manifestation of HGFmRNA in the limb bud and thorax of a nascent state mouse is shown, and light field (left-hand side) and the dark field (right-hand side) corresponding to it are photoed after in situ hybridization, an autoradiography, and dyeing. this drawing -- setting -- A and B -- the cross-section intercept of 16 age-in-day embryos' hind foot --; -- C and D show 13 age-in-day embryos, and E and F show the longitudinal-section intercept of 14 age-in-day embryos' thorax Moreover, in Ta, the tarsitis and Ti show a tibia and Rib shows precartilage nature accumulation of a rib cartilage.

[0023] As shown in drawing 1 , the diffusion manifestation of HGFmRNA was detected around [a pars-basilaris-ossis-occipitalis field] the member on the 11th. Cartilaginous accumulation was not generated in the member in this stage. Advance of cartilaginous accumulation has restricted the manifestation part of HGFmRNA more. When the stylopodium, a junction foot, and an autopodium portion were formed on the 12.5th, the manifestation of HGFmRNA was observed in the joint field of a wrist/malleolus, and an elbow/knee (refer to drawing 2 A and B.). For convenience, the knee and the malleolus were shown. HGFmRNA was discovered in the second half (13 - 14 days) into the mesenchyme system cell which cartilage accumulation of the joint field of a wrist/malleolus, and an elbow/knee adjoined, and was limited (refer to drawing 2 C-J). HGFmRNA was localized to the limited mesenchyme cell which adjoins the cartilage of the tarsitis on the 16th (refer to drawing 3 A and B). The manifestation level in the member of HGFmRNA decreased with specialization. The growth board of hand and foot did not detect HGFmRNA through the examination.

[0024] The manifestation of HGFmRNA in the thorax of the HGFmRNA manifestation nascent state mouse in the example 2 generating thorax was examined by the in situ hybridization method. The result is shown in drawing 3 C-F. As shown in drawing 3 C-F, HGFmRNA was discovered by the circumference interspace mesenchyme at the nose of cam the precartilage nature accumulation which the interspace elongated. The signal of hybridization was not detected in precartilage nature accumulation.

[0025] In order that which might decide whether to be the target cell of HGF around the cartilaginous tissue to the

chondrocyte of example 3HGF which is carrying out SUKYATTA activity test specialization, from the chondrocyte from a knee-joint cartilage and a costal cartilage, and the knee joint, the cultured cell of the synovial cell and the fibroblast increased from the member muscular system was prepared, and the effect of HGF added in external cause into these cells was examined. That is, seeding of the rabbit articular cartilage cell was carried out to the well by the density of 3x103 cells 16mm, and it maintained for two days by the culture medium A. Then, processing was performed for two days by HGF. The phase contrast microphotography was taken at the time of the end of an incubation. The result is shown in drawing 4 . As shown in drawing 4 , in HGF un-processing (control), the polygonal chondrocyte increased and it became island-like (drawing 4 A). On the other hand, in the cultivation which processed by HGF (3 ng/ml), the chondrocyte did not form an island in the state of the single cell (drawing 4 B). Therefore, it became clear that HGF stimulates movement of a chondrocyte. In addition, HGF was not distributed about the fibroblast and the synovial cell.
[0026] The effect of HGF to proliferation of the effect chondrocyte of HGF to example 4 chondrocyte proliferation was examined. That is, the articular cartilage cell extracted from the 4-weeks old rabbit was cultivated. The amount of incorporation of [3H]-thymidine was measured by the method which dealt with the cell which became confluent by HGF of various concentration, and showed it to the term of material and a method after carrying out blood serum removal processing for 24 hours. Moreover, the examination with the same said of the rabbit synovial membrane fibroblast was performed. The result is shown in drawing 5 A (articular cartilage cell) and B (synovial membrane fibroblast). In addition, a result shows the average ** standard deviation of three examinations (also setting to drawing 5 C, drawing 6 , and drawing 7 A and B the same). As shown in drawing 5 A, HGF made the incorporation of the [3H]-thymidine to a rabbit articular cartilage cell increase in dosage dependence, and having promotion of DNA synthesis, i.e., the proliferation promotion operation to an articular cartilage cell, was shown. In 1 ng/ml HGF, as for DNA synthesis, the 3 times as many increase as this was accepted to control. On the other hand, as shown in drawing 5 B, the synovial membrane fibroblast did not react to HGF.
[0027] Moreover, another experiment examined the effect of HGF to the number of cells of an articular cartilage. That is, 1x104 cell seeding of the rabbit articular cartilage cell was carried out to the well 16mm, and it maintained by the DMEM culture medium which contains FBS 10%. Subsequently, the incubation of the 10 ng/ml HGF was added and carried out for 48 hours, and the number of cells was measured after the incubation end. The result is shown in drawing 5 C. 10 ng/ml HGF made the number of cells increase by about 1.8 times compared with control, as shown in drawing 5 C.

[0028] the effect of HGF to example 5 proteoglycan generation -- as mentioned above, since HGF promoted proliferation of a chondrocyte next, the effect over proteoglycan generation of an articular cartilage cell was examined by the method shown by the above-mentioned material and the term of a method Measurement of the incorporation of the [35S]-sulfuric-acid machine to the macromolecule (glycosaminoglycan) which precipitates by the cetyl pyridinium chloride after protease digestion considered proteoglycan composition (Exp.Cell Res., 130, 73, 1980). In addition, it replaced with HGF and examined also about the following factor.
insulin Mr. growth factor (IGF)-I:concentration 100 ng/mlIGF-II:100 ng/ml concentration parathyroid-hormone (PTH): -- concentration 10-7 MTGF-beta: -- the result is shown in drawing 6 3 ng/ml concentration HGF made the incorporation of a [35S]-sulfuric-acid machine increase in dosage dependence, as shown in drawing 6 . The maximum increase was acquired by 1 ng/ml HGF. Although this operation was weaker than TGF-beta (J.Cell Physiol., 138, 329, 1989) and PTH (J.Clin.Invest., 85, 626, 1990), IGF-I and its II were of the same grade (Exp.Cell Res., 130, 73, 1980).
[0029] the effect of HGF to the DNA synthesis and proteoglycan generation under example 6 anti-HGF antibody existence -- it is thought that HGF generally acts on a target cell by the paracrine mechanism as mentioned above, and it is considered that the result of the aforementioned in situ hybridization is supporting this idea Then, in order to check this point, the HGF polyclonal antibody examined whether the function of a chondrocyte would be changed. namely, the rabbit articular cartilage cell which became confluent -- the bottom of existence of 3 ng/ml HGF or nonexistence, and an anti-[25microg //ml] HGF polyclonal antibody (IgG fraction refined by the affinity) -- processing -- or it un-processed Then, by the method shown by the aforementioned material and the term of a method, the indicator was carried out with [3H]-thymidine or the [35S]-sulfuric-acid machine, and DNA synthesis or proteoglycan generation was measured. The result is shown in drawing 7 A (DNA synthesis) and B (proteoglycan generation). In addition, in this drawing, Ab shows an anti-HGF polyclonal antibody. As shown in drawing 7 , neither the DNA synthesis in an articular cartilage cell nor proteoglycan generation was changed only by addition of an anti-HGF polyclonal antibody. However, the anti-HGF polyclonal antibody checked completely the effect of HGF added to exogenism. This shows that sufficient HGF for a chondrocyte to adjust the own function of a cartilage is not produced.
[0030] Reverse transcription PCR considered the manifestation of the HGF receptor (c-Met) in the chondrocyte besides [manifestation in-the-living-body / in the chondrocyte of the example 7HGF receptor mRNA / and] a living body. Each

portion of a joint organization and a costal cartilage was started from the 4-weeks old rat under the surgical microscope, and the total RNA was extracted as the term of a method and material described. After carrying out reverse transcription of the extracted RNA (0.5microg) and amplifying it using the primer of c-Met, it was analyzed by agarose gel electrophoresis 1.5%. The result is shown in drawing 8 . As shown in drawing 8 , the c-Met manifestation of a minute amount was detected after 40 amplification about the articular cartilage organization and the costal cartilage organization, and the cultivation chondrocyte accepted the Tsuguaki c-Met manifestation after 35 amplification.
[0031] Example of production of an example of manufacture 1HGF tablet (1) HGF 20microg human serum albumin The 100mg above-mentioned matter was dissolved by PBS of 0.01M of pH 7.0, the whole quantity was prepared to 20ml, 2ml was poured distributively into each Bayh Al bottle after sterilization, and freeze-drying seal was carried out.
(2) HGF 40microg Tween 80 1mg human serum albumin The 100mg above-mentioned matter was dissolved in the physiological saline for injection, the whole quantity was prepared to 20ml, 2ml was poured distributively into each Bayh Al bottle after sterilization, and freeze-drying seal was carried out.

[Translation done.]

*NOTICES *

1.This document has been translated by computer. So the translation may not reflect the original precisely.
2.**** shows the word which can not be translated.
3.In the drawings, any words are not translated.

DESCRIPTION OF DRAWINGS

[Brief Description of the Drawings]
[Drawing 1] It is the microphotography (gestalt of an organism) in which the manifestation of HGFmRNA in the limb bud of an early nascent state mouse is shown (light field dark field [left-hand side] corresponding to it in right-hand side). In this drawing, A-D is 10.5 age-in-day embryos, and E-H is 11 age-in-day embryos' longitudinal-section intercept.

[Drawing 2] It is the microphotography (gestalt of an organism) in which the manifestation of HGFmRNA in the limb bud of a finger formative period mouse is shown (light field dark field [left-hand side] corresponding to it in right-hand side). In this drawing, A and B are [13 age-in-day embryos and G-J of 12.5 age-in-day embryos and C-F] 14 age-in-day embryos' intercepts. Moreover, in a femur and Fi, a fibula and Ta show the tarsitis and I-V shows [Fe] a finger number.
[Drawing 3] It is the microphotography (gestalt of an organism) in which the manifestation of HGFmRNA in the limb bud and thorax of a nascent state mouse is shown (light field dark field [left-hand side] corresponding to it in right-hand side). this drawing -- setting -- A and B -- cross-section intercept [of 16 age-in-day embryos' hind foot]; -- C and D show 13 age-in-day embryos, and E and F show the longitudinal-section intercept of 14 age-in-day embryos' thorax Moreover, in Ta, the tarsitis and Ti show a tibia and Rib shows precartilage nature accumulation of a rib cartilage.
[Drawing 4] It is the microphotography (gestalt of an organism) in which the SUKYATTA activity to the chondrocyte of HGF is shown. In this drawing, A shows control (HGF un-processing) and B shows HGF processing.
[Drawing 5] It is drawing showing the effect of HGF to chondrocyte proliferation. As for A, in this drawing, B shows [C] the effect as opposed to proliferation (the number of cells) of an articular cartilage cell for the effect as opposed to the DNA synthesis of the synovial cell for the effect over the DNA synthesis of an articular cartilage cell.
[Drawing 6] It is drawing showing the effect of HGF to proteoglycan generation.
[Drawing 7] It is drawing showing the effect of HGF to the DNA synthesis (drawing 7 A) and proteoglycan generation (drawing 7 B) under anti-HGF antibody existence.
[Drawing 8] It is the electrophoresis photograph in which the manifestation by the chondrocyte of the HGF receptor mRNA is shown.

[Translation done.]

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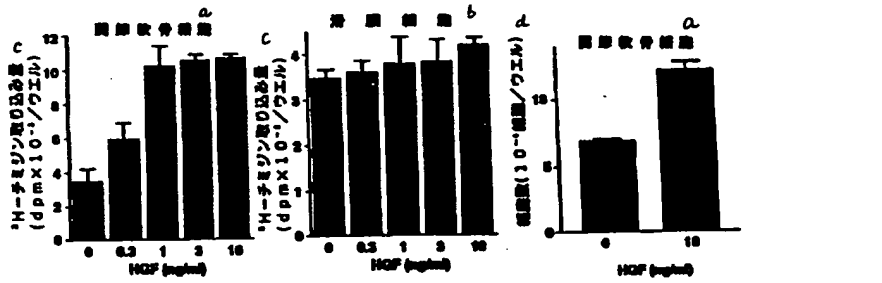


(57) Abstract

A cartilage disease remedy, cartilage cell growth accelerator and proteoglycan formation accelerator, each containing a hepatocyte growth factor (HGF) as the active ingredient, and a method of treating human and mammalian cartilage diseases by administering an effective dose f HGF. The HGF as the active ingredient accelerates cartilage cell growth and proteoglycan formation. Therefore the remedy and accelerators are useful for preventing and treating various cartilage diseases.

(57) 要約

本発明は、ＨＧＦ（肝細胞増殖因子）を有効成分として含有することからなる軟骨障害治療剤、軟骨細胞増殖促進剤及びプロテオグリカン生成促進剤並びに有効量のＨＧＦを投与することからなるヒト及び哺乳動物の軟骨障害の治療法に関する。有効成分であるＨＧＦは、軟骨細胞の増殖を促進し、プロテオグリカンの生成を促進する作用を有する。従って、本発明の治療剤及び促進剤は、各種の軟骨障害疾患の予防・治療に有用である。

明 細 書

軟骨障害治療剤

技術分野

本発明は軟骨疾患の治療及び予防に有用な薬剤に関する。より詳細には、ＨＧＦ(Hepatocyte Growth Factor)を有効成分として含有する軟骨障害治療剤、軟骨細胞増殖促進剤及びプロテオグリカン生成促進剤に関する。

背景技術

軟骨は軟骨細胞とこれを取り囲む基質からなる結合組織であり、関節、脊柱の椎間板、肋軟骨、耳介、外耳道、恥骨結合、咽喉蓋などに存在する。軟骨は、軟骨細胞と、軟骨細胞が産生する軟骨基質からなり、軟骨基質はコラーゲン線維などの線維成分、プロテオグリカン及び水が主な成分であり、軟骨は軟骨基質の混じりぐあいにより、硝子軟骨（肋軟骨、咽喉軟骨、関節軟骨など）、弾性軟骨（耳介軟骨など）及び線維軟骨（椎間板軟骨、恥骨軟骨、関節軟骨など）に分類することができる。上記の軟骨基質において、コラーゲン線維は軟骨の張力及び剪断力に対する剛性と強度に関与し、プロテオグリカンは圧縮力に対する強度に関与し、水は生体組織の粘弾性体としての特性にあずかっているとされており、例えば、関節軟骨の場合、軟骨の質重量の７８．６％は水が占め、コラーゲンが２０％を占め、プロテオグリカンが７％を占めている。

軟骨の作用としては、骨端の摩擦の低減（骨間の軟骨）、弾性の保持（耳介軟骨など）、運動機能（肋軟骨、恥骨軟骨など）が挙げられる。

上記のように軟骨は生体の機能維持の上で重要な作用を有しており、従来から軟骨の障害に起因する種々の疾患が知られ、例えば、軟骨形成異常症、変形性関節症、変形性椎間板症、骨折の修復・治癒不全などが例示される。特に、高齢化社会の到来、スポーツによる外傷の増加、キーパンチャー病などに代表される職業病の出現などにより、関節障害患

者は著しく増加しており、この領域における医療の進歩が要望されている。

従来から軟骨障害を治療するために種々の治療法が試みられてきているが、それらは直接的に原因の解消を目的とするものではなく、例えば、抗炎症剤などを投与することにより、その疾患に基づく痛みなどの障害を抑制する方法；関節にヒアルロン酸製剤などを注入して関節の動きを潤滑にする方法など、対症療法的なものでしかなかった。

このように、関節障害の根治的治療法は見出されておらず、特に変形性関節症は患者数が多く、その有効な治療法が切望されている。

本発明者らは上記の課題を解決するために鋭意検討した結果、ＨＧＦが軟骨細胞の増殖を促進し、またプロテオグリカンの生成を促進する作用を有し、軟骨障害に起因する種々の疾患の治療に有効であることを見出し、本発明を完成させた。

上記のＨＧＦは肝実質細胞を in vitro で増殖させる因子として見出されたタンパク質である(Biochem Biophys Res Commun, 122, 1450, 1984、Proc. Natl. Acad. Sci. USA, 83, 6489, 1986、FEBS Letter, 224, 311, 1987、Nature, 342, 440, 1989、Proc. Natl. Acad. Sci. USA, 87, 3200, 1990）。肝実質細胞を特異的に増殖させる因子として発見されたＨＧＦは、本発明者らをはじめとする多くの研究者による最近の研究成果によって、生体内で組織傷害治癒などの種々の活性を示している事が明らかとなり、研究対象としてのみならずヒトや動物の治療薬などへの応用に期待が集まっている。

ＨＧＦは主に間葉系の細胞により産生されていることが解明されており、近隣細胞から必要に応じてＨＧＦが供給される、所謂パラクリン機構が成立していることが明らかにされている。しかしながら、肝臓や腎臓に傷害を受けたとき、傷害を受けていない臓器、例えば肺などにおいてもＨＧＦの産生が高まることから、所謂エンドクリン機構によってもＨＧＦが供給されていると考えられる。

このようなＨＧＦの受容体に関して、最近の研究から、ｃ－Ｍｅｔ原腫瘍遺伝子がＨＧＦ受容体をコードしていることが確定的になった(Bot

taro et al., Science 251, 802-804, 1991; Naldini et al., Oncogene 6, 501-504, 1991)。

上述のようにＨＧＦに関しては多くの知見が得られているが、ＨＧＦの軟骨細胞増殖促進作用及びプロテオグリカン生成促進作用は従来知られていない新規な知見であり、かかる知見に基づいてなされた本発明は軟骨障害に起因する種々の疾患の治療に有用な薬剤を提供することにある。

発明の開示

本発明は、ＨＧＦを有効成分として含有することからなる軟骨障害治療剤である。

また、本発明の他の発明は、ＨＧＦを有効成分として含有することからなる軟骨細胞増殖促進剤；ＨＧＦを有効成分として含有することからなるプロテオグリカン生成促進剤；有効量のＨＧＦを投与することからなるヒト又は哺乳動物の軟骨障害の治療法である。

上記のＨＧＦは、ヒト又は動物の組織又は血液成分由来のものであってもよく、また遺伝子組換えにより製造したものであってもよい。

有効成分であるＨＧＦは、軟骨細胞の増殖を促進し、プロテオグリカンの生成を促進する作用を有するので、軟骨障害に起因する種々の疾患の治療・予防に有効である。

図面の簡単な説明

図１は、早期発生期マウスの肢芽におけるＨＧＦｍＲＮＡの発現を示す顕微鏡写真である（左側は明視野、右側はそれに対応する暗視野）。同図において、Ａ～Ｄは１０．５日齢胎児、Ｅ～Ｈは１１日齢胎児の縦断面切片である。

図２は、指形成期マウスの肢芽におけるＨＧＦｍＲＮＡの発現を示す顕微鏡写真である（左側は明視野、右側はそれに対応する暗視野）。同図において、Ａ及びＢは１２．５日齢胎児、Ｃ～Ｆは１３日齢胎児、Ｇ～Ｊは１４日齢胎児の切片である。また、Ｆｅは大腿骨、Ｆｉは腓骨、

Ｔａは足根骨を、Ｉ～Ｖは指番号を示す。

図３は、発生期マウスの肢芽及び胸郭におけるＨＧＦｍＲＮＡの発現を示す顕微鏡写真である（左側は明視野、右側はそれに対応する暗視野）。同図において、Ａ及びＢは１６日齢胎児の後肢の横断面切片；Ｃ及びＤは１３日齢胎児、Ｅ及びＦは１４日齢胎児の胸郭の縦断面切片を示す。また、Ｔａは足根骨、Ｔｉは脛骨、Ｒｉｂは肋骨軟骨の前軟骨性集積を示す。

図４は、ＨＧＦの軟骨細胞に対するスキャッター活性を示す顕微鏡写真である。同図において、Ａはコントロール（ＨＧＦ非処理）を、ＢはＨＧＦ処理を示す。

図５は、軟骨細胞増殖に対するＨＧＦの効果を示す図である。同図において、Ａは関節軟骨細胞のＤＮＡ合成に対する効果を、Ｂは滑膜細胞のＤＮＡ合成に対する効果を、Ｃは関節軟骨細胞の増殖（細胞数）に対する効果を示す。

図６は、プロテオグリカン生成に対するＨＧＦの効果を示す図である。

図７は、抗ＨＧＦ抗体存在下における、ＤＮＡ合成（図７Ａ）及びプロテオグリカン生成（図７Ｂ）に対するＨＧＦの効果を示す図である。

図８は、ＨＧＦレセプターｍＲＮＡの軟骨細胞での発現を示す電気泳動写真である。

発明を実施するための最良の形態

本発明で使用されるＨＧＦとしては、医薬として使用できる程度に精製されたものであれば、種々の方法で調製されたものを用いることができる。

ＨＧＦの調製方法としては、各種の方法が知られている。例えば、ラット、ウシ、ウマ、ヒツジなどの哺乳動物の肝臓、脾臓、肺臓、骨髄、脳、腎臓、胎盤等の臓器、血小板、白血球等の血液細胞や血漿、血清などから抽出、精製して得ることができる(FEBS Letter, 224, 312, 1987、Proc. Natl. Acad. Sci. USA, 86, 5844, 1989など参照)。

また、ＨＧＦを産生する初代培養細胞や株化細胞を培養し、培養物

（培養上清、培養細胞等）から分離精製してＨＧＦを得ることもできる。あるいは遺伝子工学的手法によりＨＧＦをコードする遺伝子を適切なベクターに組込み、これを適当な宿主に挿入して形質転換し、この形質転換体の培養上清から目的とする組換えＨＧＦを得ることができる(例えば、Nature, 342, 440, 1989、日本国特開平５－１１１３８３号公報、Biochem. Biophys. Res. Commun., 163, 967, 1989など参照)。上記の宿主細胞は特に限定されず、従来から遺伝子工学的手法で用いられている各種の宿主細胞、例えば大腸菌、枯草菌、酵母、糸状菌、植物又は動物細胞などを用いることができる。

より具体的には、ＨＧＦを生体組織から抽出精製する方法としては、例えば、ラットに四塩化炭素を腹腔内投与し、肝炎状態にしたラットの肝臓を摘出して粉砕し、Ｓ－セファロース、ヘパリンセファロースなどのゲルカラムクロマトグラフィー、ＨＰＬＣ等の通常の蛋白質精製法にて精製することができる。

また、遺伝子組換え法を用い、ヒトＨＧＦのアミノ酸配列をコードする遺伝子を、ウシパピローマウィルスＤＮＡなどのベクターに組み込んだ発現ベクターによって動物細胞、例えば、チャイニーズハムスター卵巣（ＣＨＯ）細胞、マウスＣ１２７細胞、サルＣＯＳ細胞などを形質転換し、その培養上清より得ることができる。

かくして得られたＨＧＦは、ＨＧＦと実質的に同効である限り、そのアミノ酸配列の一部が欠失又は他のアミノ酸により置換されていたり、他のアミノ酸配列が一部挿入されていたり、Ｎ末端及び／又はＣ末端に１又は２以上のアミノ酸が結合していたり、あるいは糖鎖が同様に欠失又は置換されていてもよい。

本発明の治療剤及び促進剤は上記のＨＧＦを有効成分とし、ＨＧＦは後記試験例に示されるように、軟骨細胞の増殖を促進し、またプロテオグリカンの生成を促進する作用を有する。更に、ＨＧＦは、障害を受けていない軟骨組織には作用を示さず、障害を有する軟骨組織にのみ作用するので、副作用を惹起するおそれが少ないという特長を有する。従って、本発明の治療剤及び促進剤は、軟骨障害に起因する各種疾患の治療・

予防に有効であり、これらには例えば下記の疾患が包含される。
変形性関節炎
軟骨形成異常症
骨折の治癒及び修復
外傷による関節軟骨、関節円板の修復
急性化膿性関節炎
結核性関節炎
梅毒性関節炎
慢性関節リウマチ
リウマチ熱、全身性エリテマトーデス
変形性脊椎症
椎間板ヘルニア
骨移植による修復

本発明の治療剤及び促進剤は、ヒトの他、哺乳動物（例えば、ウシ、ウマ、ブタ、ヒツジ、イヌ、ネコ等）における軟骨障害に起因する種々の疾患の治療・予防に適用される。

本発明の治療剤及び促進剤は種々の製剤形態（例えば、液剤、固形剤、カプセル剤等）をとりうるが、一般的には有効成分であるＨＧＦのみ又はそれと慣用の担体と共に注射剤、吸入剤、坐剤又は経口剤とされる。当該注射剤は常法により調製することができ、例えば、ＨＧＦを適切な溶剤（例えば、滅菌された水、緩衝液、生理食塩水等）に溶解した後、フィルター等で濾過して滅菌し、次いで無菌的な容器に充填することにより調製することができる。注射剤中のＨＧＦ含量としては、通常0.0002～0.2(W/V%)程度、好ましくは0.001～0.1(W/V%)程度に調整される。また、経口薬としては、例えば、錠剤、顆粒剤、細粒剤、散剤、軟又は硬カプセル剤、液剤、乳剤、懸濁剤、シロップ剤などの剤形に製剤化され、これらの製剤は製剤化の常法に準じて調製することができる。坐剤も慣用の基剤（例えば、カカオ脂、ラウリン脂、グリセロゼラチン、マクロゴール、ウィテップゾル等）を用いた製剤上の常法によって調製することができる。また、吸入剤も製剤上の常套手段に準じて調製するこ

とができる。

製剤中のＨＧＦ含量は、剤形、適用疾患などに応じて適宜調整することができる。

製剤化に際して、好ましくは安定化剤が添加され、安定化剤としては、例えば、アルブミン、グロブリン、ゼラチン、グリシン、マンニトール、グルコース、デキストラン、ソルビトール、エチレングリコールなどが挙げられる。さらに、本発明の製剤は製剤化に必要な添加物、例えば、賦形剤、溶解補助剤、酸化防止剤、無痛化剤、等張化剤等を含んでいてもよい。液状製剤とした場合は凍結保存、又は凍結乾燥等により水分を除去して保存するのが望ましい。凍結乾燥製剤は、用時に注射用蒸留水などを加え、再溶解して使用される。

本発明の治療剤及び促進剤は、その製剤形態に応じた適当な投与経路により投与され得る。例えば、注射剤の形態にして静脈、動脈、皮下、筋肉内などに投与することができる。その投与量は、患者の症状、年齢、体重などにより適宜調整されるが、通常ＨＧＦとして0.05mg～500mg、好ましくは1mg～100mgであり、これを１日１回ないし数回に分けて投与するのが適当である。

産業上の利用可能性

本発明において、有効成分であるＨＧＦは、軟骨細胞の増殖を促進し、またプリテオグリカンの生成を促進させる作用を有している。従って、本発明の治療剤及び促進剤は、前述した軟骨障害に起因する各種疾患の治療・予防に有用である。更に、ＨＧＦは、障害を受けている軟骨組織にのみ作用するので、副作用の少ない薬剤を得ることができるという効果を奏する。

実施例

以下、実施例及び製造例に基づいて本発明をより詳細に説明するが、本発明はこれらの例に限定されるものではない。なお、以下の実験で使用した材料及び方法は以下のとおりである。

材料と方法

①in situハイブリダイゼーション

ラットＨＧＦ－ｃＤＮＡ（ＲＢＣ１　クローン）(Proc. Natl. Acad. Sci. USA, 87, 3200, 1990)の１．４ｋｂ　ＥｃｏＲＩ断片をｐＧＥＭ７ベクターにサブクローンし［ａ－^{35}Ｓ］ＵＴＰ（４００Ｃｉ／ｍｍｏｌ、アマシャム社）で標識化したアンチセンスとセンスのＲＮＡプローブを作製した。標識した転写物は、リボプローブとして５０－１５０塩基にアルカリ加水分解した。

in situハイブリダイゼーションは文献(Biochem. Biophys. Res. Commun., 173, 42, 1990)記載の方法で実施した。サンプルは４％パラホルムアルデヒドーリン酸生理食塩水溶液で固定し、エタノールで脱水、トルエンで洗浄後、パラフィンに包埋した。５μｍの切片を切り出し、ポリーＬ－リジンでコートしたスライドグラスにマウントした。切片はグリシンと無水酢酸で脱パラフィンし、５０℃、１６時間プローブでハイブリダイズした。その後、切片を０．１×ＳＳＣ液で５０℃、１時間洗浄し、ＲＮＡａｓｅＡ（２０μｇ／ｍｌ）で３７℃、３０分処理してから、２×ＳＳＣ液で３７℃、１０分間で２回洗浄した。切片は乳剤（１：１コダックＮＢＴ－２希釈液）に浸し２週間露光した。切片はコダックＤ－１９に現像定着し、ヘマトキシリン・エオジンで染色した。

②細胞と細胞培養

軟骨細胞は、文献(J. Cell. Physiol., 133, 491, 1987)記載の方法に準じて、ニュージーランドホワイトウサギの２３日齢胎児と４週齢新生児から単離した。関節軟骨は膝の大腿骨関節軟骨から、肋軟骨は肋骨の硝子軟骨から単離した(Dev. Biol., 136, 500, 1989)。滑膜線維芽細胞は、膝関節の滑膜組織から単離した。細切した滑膜組織断片を１０％ＦＢＳを含むＤＭＥＭで１０日間培養し、トリプシン処理で増殖した細胞を集めた。文献(Exp. Cell Res., 157, 483, 1985)記載の方法に準じて、２０日齢ラット胎児の脚筋肉組織から胎生間葉系細胞を単離した。脚芽間葉細胞は、１０．５日齢のラット胎児から単離した。脚芽は外科

用顕微鏡下で切り出し、０．２５％トリプシンで３０分で処理後、ピペッテングしナイロンガーゼで単離細胞を得た。脚芽細胞以外全ての細胞は、１０％ＦＢＳ、６０μｇ／ｍｌのカナマイシンを含むＤＭＥＭ（以下、培地Ａという）で３７℃、５％CO_2／９５％空気下で維持した。

③ＤＮＡ合成の測定

ＤＮＡ合成速度は、１０％ＴＣＡ不溶性細胞沈殿への［^{3}Ｈ］－チミジン（［６－^{3}Ｈ］－チミジン、アマシャム社、２０Ｃｉ／ｍｍｏｌ）の取り込みの測定で評価した(J. Clin. Invest., 85, 626, 1990)。細胞は、９６穴プレートの６ｍｍウエル当り１．５×１０^{4}個の密度で播種し、コンフルエントになるまで培養した。増殖を停止するため、細胞は０．３％ＦＢＳ含有ＤＭＥＭの０．１ｍｌでプレインキュベーションした。種々の濃度のＨＧＦを培地に添加した。インキュベーションは２４時間続けた。１μＣｉ／ｍｌ［^{3}Ｈ］－チミジンは、インキュベーション停止３時間前に添加した。標識後細胞は氷冷ＰＢＳで３回、３ｍＭチミジンを含有する５％ＴＣＡで２回、エタノール：ジメチルエーテル（３：１）で１回洗浄した。ウエル内の残渣は、１００μｌの０．１Ｎ ＮａＯＨで可溶化し、液体シンチバイアルに移し、１Ｎ ＨＣｌで中和後、放射能をシンチレーションカウンター（Ｒａｃｋ－ｂｅｔａ、ファルマシア社）で測定した。

④プロテオグリカン合成の測定

軟骨細胞は、６ｍｍウエル当り１．５×１０^{4}個の密度で播種し、０．１ｍｌの培地Ａで維持した。細胞がコンフルエントに達したら、０．３％ＦＢＳを含有する０．１ｍｌのＤＭＥＭで２４時間プレインキュベーションした。その後、０．３％ＦＢＳとＨＧＦを含有する０．１ｍｌのＤＭＥＭで２４時間インキュベーションした。１μＣｉ／ｍｌの［^{35}Ｓ］－硫酸基をインキュベーション終了２０時間前に添加した。プロテオグリカン合成は、プロテアーゼ消化後のセチルピリジニウムクロライドでの沈殿物への［^{35}Ｓ］－硫酸基の取り込みの測定により評価した(Exp.

Cell Res., 130, 73, 1980)。

⑤総ＲＮＡ調製と逆転写ＰＣＲ

軟骨から総ＲＮＡは、文献(Anal. Biochem., 203, 352, 1992)記載の方法の変法で調製した。新鮮単離組織断片（０．１ｇ湿重量）は、４Ｍグアニジンチオシアネート、０．１Ｍ　Ｔｒｉｓ塩酸（ｐＨ７．５）、１％２－メルカプトエタノールの４Ｍ　ＧＩＴＣ溶液２ｍｌですばやくホモジェネートした。ホモジェネートは１０％ＳＤＳ１００μｌに混合し、微量遠心機で５分間遠心した。上清２ｍｌをベックマンポリアロマー遠心チューブ（１３×５１ｍｍ）中で、同容量の１．６ｇセシウムトリフルオロアセテートと１ｍＭ　ＥＤＴＡ（ｐＨ８．０）に重層した。試料を３５，０００ｒｐｍ（１４７，０００×ｇ）で１８℃、２０時間遠心した。上清を吸引除去後、沈殿を４Ｍ　ＧＩＴＣ溶液２００μｌに溶解し、フェノール：クロロホルム：イソアミルアルコール（２５：２４：１）で抽出処理後、２０μｌの３Ｍ酢酸ナトリウム（ｐＨ４．８）を混ぜ、２倍容量（４４０μｌ）のエタノールで沈殿させた。沈渣はＤＥＰＣ処理水に溶解した。

まず、０．５μｇ総ＲＮＡからファーストストランドｃＤＮＡの合成をＳｕｐｅｒＳｃｒｉｐｔ逆転酵素(Gibco-BRL)と下流域のアンチセンスプライマーを使って行った。引き続いてＰＣＲ増殖を行った。増殖は、９４℃で３０秒、５８℃で１分、７２℃で１．５分での３５サイクル（軟骨細胞の場合）又は４０サイクル（軟骨組織の場合）の条件で行った。ＰＣＲ増殖のプライマー塩基配列は、ラットとマウスのｃ－Ｍｅｔ(Oncogene, 2, 593, 1988)に対しては5’－ＣＡＧＴ（Ａ／Ｇ）ＡＴＧＡＴＣＴＣＡＡＴＧＧＧＣＡＡＴ－3’と5’－ＡＡＴＧＣＣＣＴＣＴＴＣＣＴＡＴＧＡＣＴＴＣ－3’で７２５ｂｐ断片を作製した。

実施例１

発生四肢でのＨＧＦｍＲＮＡ発現

発生期マウスの肢芽におけるＨＧＦｍＲＮＡの発現をin situハイブ

リダイゼーション法で試験した。その結果を図１、図２及び図３に示す。

図１は早期発生期マウスの肢芽におけるＨＧＦｍＲＮＡの発現を示し、後肢の縦断面切片の顕微鏡写真であり、明視野（左側）及びそれに対応する暗視野（右側）はin situハイブリダイゼーション、オートラジオグラフィ及び染色後に撮影したものである。同図において、Ａ～Ｄは１０．５日齢胎児、Ｅ～Ｈは１１日齢胎児の切片である。

図２は指形成期マウスの肢芽におけるＨＧＦｍＲＮＡの発現を示し、後肢の縦断面切片の顕微鏡写真であり、明視野（左側）及びそれに対応する暗視野（右側）はin situハイブリダイゼーション、オートラジオグラフィ及び染色後に撮影したものである。同図において、Ａ及びＢは１２．５日齢胎児、Ｃ～Ｆは１３日齢胎児、Ｇ～Ｊは１４日齢胎児の切片である。また、Ｆｅは大腿骨、Ｆｉは腓骨、Ｔａは足根骨を、Ｉ～Ｖは指番号を示す。

図３は発生期マウスの肢芽及び胸郭におけるＨＧＦｍＲＮＡの発現を示す顕微鏡写真であり、明視野（左側）及びそれに対応する暗視野（右側）はin situハイブリダイゼーション、オートラジオグラフィ及び染色後に撮影したものである。同図において、Ａ及びＢは１６日齢胎児の後肢の横断面切片を；Ｃ及びＤは１３日齢胎児、Ｅ及びＦは１４日齢胎児の胸郭の縦断面切片を示す。また、Ｔａは足根骨、Ｔｉは脛骨、Ｒｉｂは肋骨軟骨の前軟骨性集積を示す。

図１に示されるように、１１日目に四肢の底部領域周囲にＨＧＦｍＲＮＡのびまん性の発現が検出された。この段階で軟骨性集積は、四肢に発生していなかった。軟骨性集積が進行するとＨＧＦｍＲＮＡの発現部位はより制限されてきた。１２．５日目に基脚、接合脚、自脚部分が形成されるとき、ＨＧＦｍＲＮＡの発現は、手首／踝と肘／膝の関節領域で観察された（図２Ａ及びＢ参照。便宜上、膝及び踝について示した）。後期（１３～１４日）には、ＨＧＦｍＲＮＡは、手首／踝と肘／膝の関節領域の軟骨集積の隣接し限定された間葉系細胞に発現していた（図２Ｃ～Ｊ参照）。１６日目に、ＨＧＦｍＲＮＡは、足根骨の軟骨に隣接する限定された間葉細胞に局在化されていた（図３Ａ及びＢ参照）。ＨＧ

ＦｍＲＮＡの四肢での発現レベルは、分化と共に減少した。試験を通じて手足の成長板でＨＧＦｍＲＮＡを検出しなかった。

実施例２

発生胸郭でのＨＧＦｍＲＮＡ発現

発生期マウスの胸郭におけるＨＧＦｍＲＮＡの発現をin situハイブリダイゼーション法で試験した。その結果を図３Ｃ～Ｆに示す。

図３Ｃ～Ｆに示されるように、ＨＧＦｍＲＮＡは、肋間の伸長した前軟骨性集積の先端の周囲肋間間葉組織で発現していた。前軟骨性集積ではハイブリダイゼーションのシグナルは、検出されなかった。

実施例３

ＨＧＦの軟骨細胞に対するスキャッター活性試験

分化している軟骨組織の周囲でどれがＨＧＦの標的細胞かを決めるため、膝関節軟骨と肋軟骨からの軟骨細胞、膝関節から滑膜細胞、四肢筋肉組織から増殖した線維芽細胞の培養細胞を調製し、これらの細胞に外因的に添加したＨＧＦの効果を検討した。

即ち、ウサギ関節軟骨細胞を１６ｍｍウエルに３×１０^{5}細胞の密度で播種し、培地Ａで２日間維持した。その後、ＨＧＦで２日間処理を行った。インキュベーションの終了時に、位相差顕微鏡写真を撮影した。その結果を図４に示す。

図４に示されるように、ＨＧＦ非処理（コントロール）においては、多角形の軟骨細胞が増殖し島状になった（図４Ａ）。一方、ＨＧＦ（３ｎｇ／ｍｌ）で処理をした培養では軟骨細胞は、単細胞状態で島を形成しなかった（図４Ｂ）。従って、ＨＧＦは軟骨細胞の移動を刺激することが明らかになった。なお、ＨＧＦは、線維芽細胞及び滑膜細胞については分散させなかった。

実施例４

軟骨細胞増殖に対するＨＧＦの効果

軟骨細胞の増殖に対するＨＧＦの効果を検討した。

即ち、４週齢のウサギから採取した関節軟骨細胞を培養した。コンフルエントになった細胞を２４時間血清除去処理をした後、種々の濃度のＨＧＦで処置し、材料及び方法の項に示した方法により［^{3}Ｈ］－チミジンの取り込み量を測定した。また、ウサギ滑膜線維芽細胞についても同様な試験を行った。その結果を図５Ａ(関節軟骨細胞)及びＢ(滑膜線維芽細胞)に示す。なお、結果は３回の試験の平均値±標準偏差を示す(図５Ｃ、図６並びに図７Ａ及びＢにおいても同様)。

図５Ａに示されるように、ＨＧＦは、ウサギ関節軟骨細胞への［^{3}Ｈ］－チミジンの取り込みを用量依存的に増加させ、ＤＮＡ合成の促進、即ち関節軟骨細胞に対する増殖促進作用を有することが示された。ＤＮＡ合成は、１ｎｇ／ｍｌのＨＧＦにおいて、コントロールに対して３倍の増加が認められた。一方、図５Ｂに示されるように、滑膜線維芽細胞はＨＧＦに反応しなかった。

また、別実験で、関節軟骨の細胞数に対するＨＧＦの効果を検討した。即ち、１６ｍｍウエルにウサギ関節軟骨細胞を１×１０^{5}細胞播種し、１０％ＦＢＳを含むＤＭＥＭ培地で維持した。次いで、１０ｎｇ／ｍｌのＨＧＦを添加して４８時間インキュベーションし、インキュベーション終了後、細胞数を測定した。その結果を図５Ｃに示す。

図５Ｃに示されるように、１０ｎｇ／ｍｌのＨＧＦは、コントロールに比べて細胞数を約１．８倍増加させた。

実施例５

プロテオグリカン生成に対するＨＧＦの効果

上記のように、ＨＧＦは軟骨細胞の増殖を促進したので、次に、前述の材料及び方法の項で示した方法により、関節軟骨細胞のプロテオグリカン生成に対する効果を検討した。プロテオグリカン合成は、プロテアーゼ消化後セチルピリジニウムクロライドで沈殿する巨大分子（グリコサミノグリカン）への［^{35}Ｓ］－硫酸基の取り込みの測定で検討した(Exp. Cell Res., 130, 73, 1980)。なお、ＨＧＦに代えて、下記の因子

についても試験した。

インスリン様成長因子（ＩＧＦ）－Ｉ：濃度１００ｎｇ／ｍｌ

ＩＧＦ－ＩＩ：濃度１００ｎｇ／ｍｌ

副甲状腺ホルモン（ＰＴＨ）：濃度10^{-7}Ｍ

ＴＧＦ－β：濃度３ｎｇ／ｍｌ

その結果を図６に示す。図６に示されるように、ＨＧＦは、用量依存的に［^{35}Ｓ］－硫酸基の取り込みを増加させた。最大増加は、１ｎｇ／ｍｌのＨＧＦで得られた。この作用は、ＴＧＦ－β(J. Cell Physiol., 138, 329, 1989)やＰＴＨ(J. Clin. Invest., 85, 626, 1990)よりは弱かったが、ＩＧＦ－Ｉ及びＩＩとは同程度であった(Exp. Cell Res., 130, 73, 1980)。

実施例６

抗ＨＧＦ抗体存在下における、ＤＮＡ合成及びプロテオグリカン生成に対するＨＧＦの効果

前述のように、一般にＨＧＦはパラクリン機構で標的細胞に作用すると考えられており、前記のin situハイブリダイゼーションの結果は、この考えを支持していると思料される。そこで、この点を確認するために、ＨＧＦポリクローナル抗体が、軟骨細胞の機能を変化させるかどうかを検討した。

即ち、コンフルエントになったウサギ関節軟骨細胞を、３ｎｇ／ｍｌのＨＧＦの存在下又は非存在下、２５μｇ／ｍｌの抗ＨＧＦポリクローナル抗体（アフィニティーで精製したＩｇＧ画分）で処理又は非処理した。その後、前記の材料及び方法の項で示した方法により、［^{3}Ｈ］－チミジン又は［^{35}Ｓ］－硫酸基で標識し、ＤＮＡ合成又はプロテオグリカン生成を測定した。その結果を図７Ａ（ＤＮＡ合成）及びＢ（プロテオグリカン生成）に示す。なお、同図において、Ａｂは抗ＨＧＦポリクローナル抗体を示す。

図７に示されるように、抗ＨＧＦポリクローナル抗体の添加だけでは、関節軟骨細胞でのＤＮＡ合成もプロテオグリカン生成も変化させなかっ

た。しかしながら、抗ＨＧＦポリクローナル抗体は、外因性に添加したＨＧＦの効果を完全に阻害した。このことは、軟骨細胞が軟骨自身の機能を調節するのに十分なＨＧＦを産生していないことを示している。

実施例７

ＨＧＦレセプターｍＲＮＡの軟骨細胞での発現

生体内及び生体外における軟骨細胞でのＨＧＦレセプター（ｃ－Ｍｅｔ）の発現を逆転写ＰＣＲで検討した。関節組織と肋軟骨の各部分を外科用顕微鏡下で、４週齢ラットから切り出し、総ＲＮＡは、方法と材料の項で述べたように抽出した。抽出したＲＮＡ（０．５μｇ）は逆転写し、ｃ－Ｍｅｔのプライマーを用いて増幅した後、１．５％アガロースゲル電気泳動法により分析した。その結果を図８に示す。

図８に示されるように、関節軟骨組織及び肋軟骨組織について４０回の増幅後、微量のｃ－Ｍｅｔ発現を検出し、培養軟骨細胞は３５回の増幅後、著明なｃ－Ｍｅｔ発現を認めた。

製造例１

ＨＧＦ製剤の生産例

(1) ＨＧＦ　２０μｇ
ヒト血清アルブミン　１００ｍｇ

上記物質をｐＨ７．０の０．０１ＭのＰＢＳで溶解し、全量を２０ｍｌに調製し、滅菌後、バイアル瓶に２ｍｌずつ分注し、凍結乾燥密封した。

(2) ＨＧＦ　４０μｇ
ツイーン８０　１ｍｇ
ヒト血清アルブミン　１００ｍｇ

上記物質を注射用生理食塩水に溶解し、全量を２０ｍｌに調製し、滅菌後、バイアル瓶に２ｍｌずつ分注し、凍結乾燥密封した。

請 求 の 範 囲

1．ＨＧＦを有効成分として含有することからなる軟骨障害治療剤。

2．ＨＧＦが遺伝子組換えにより製造したものである請求の範囲第１項に記載の軟骨障害治療剤。

3．軟骨障害が、変形性関節炎、軟骨形成異常症、骨折の治癒及び修復、外傷による関節軟骨及び関節円板の修復、急性化膿性関節炎、結核性関節炎、梅毒性関節炎、慢性関節リウマチ、リウマチ熱、全身性エリテマトーデス、変形性脊椎症、椎間板ヘルニア又は骨移植による修復である請求の範囲第１項又は第２項に記載の軟骨障害治療剤。

4．ＨＧＦを有効成分として含有することからなる軟骨細胞増殖促進剤。

5．ＨＧＦを有効成分として含有することからなるプロテオグリカン生成促進剤。

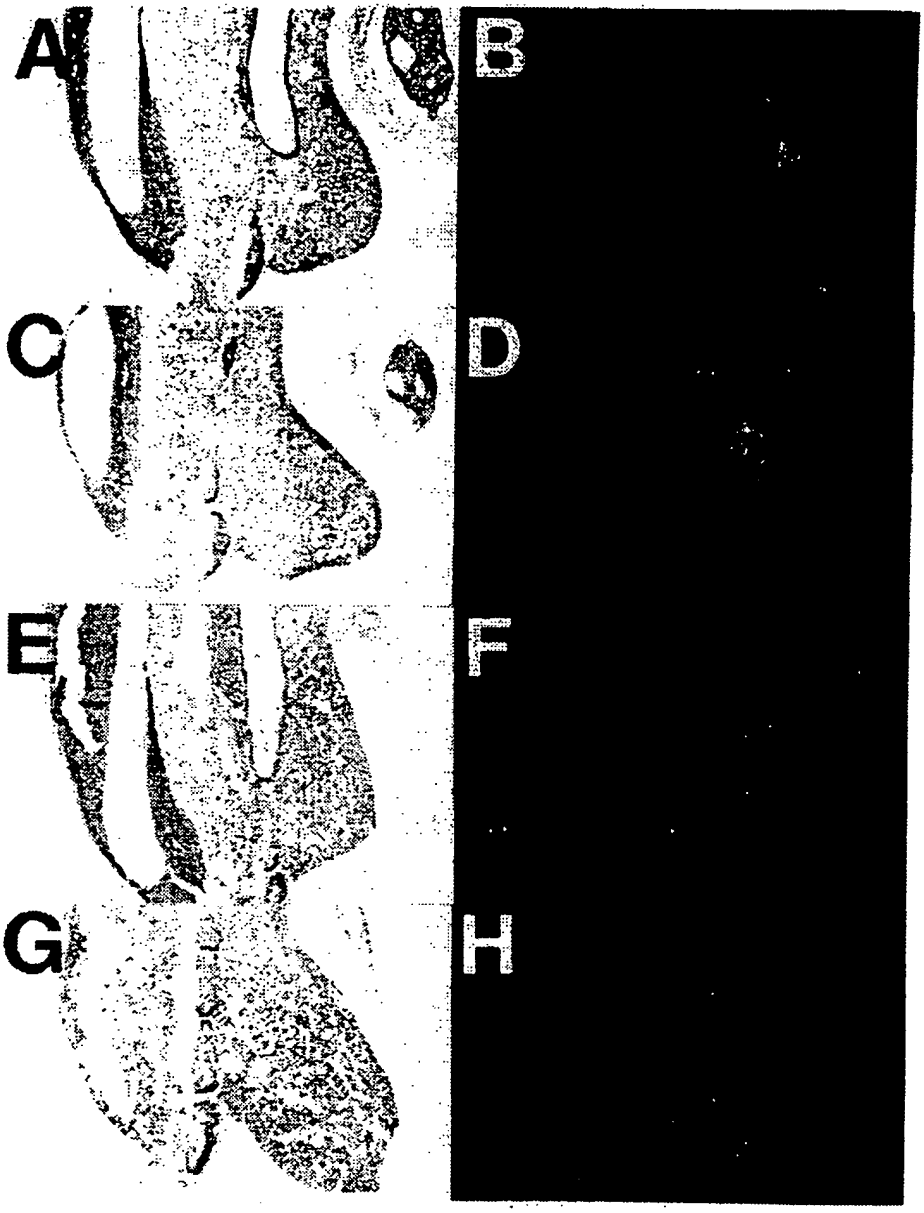
6．ＨＧＦが遺伝子組換えにより製造したものである請求の範囲第４項又は第５項に記載の剤。

7．有効量のＨＧＦを投与することからなるヒト又は哺乳動物の軟骨障害の治療法。

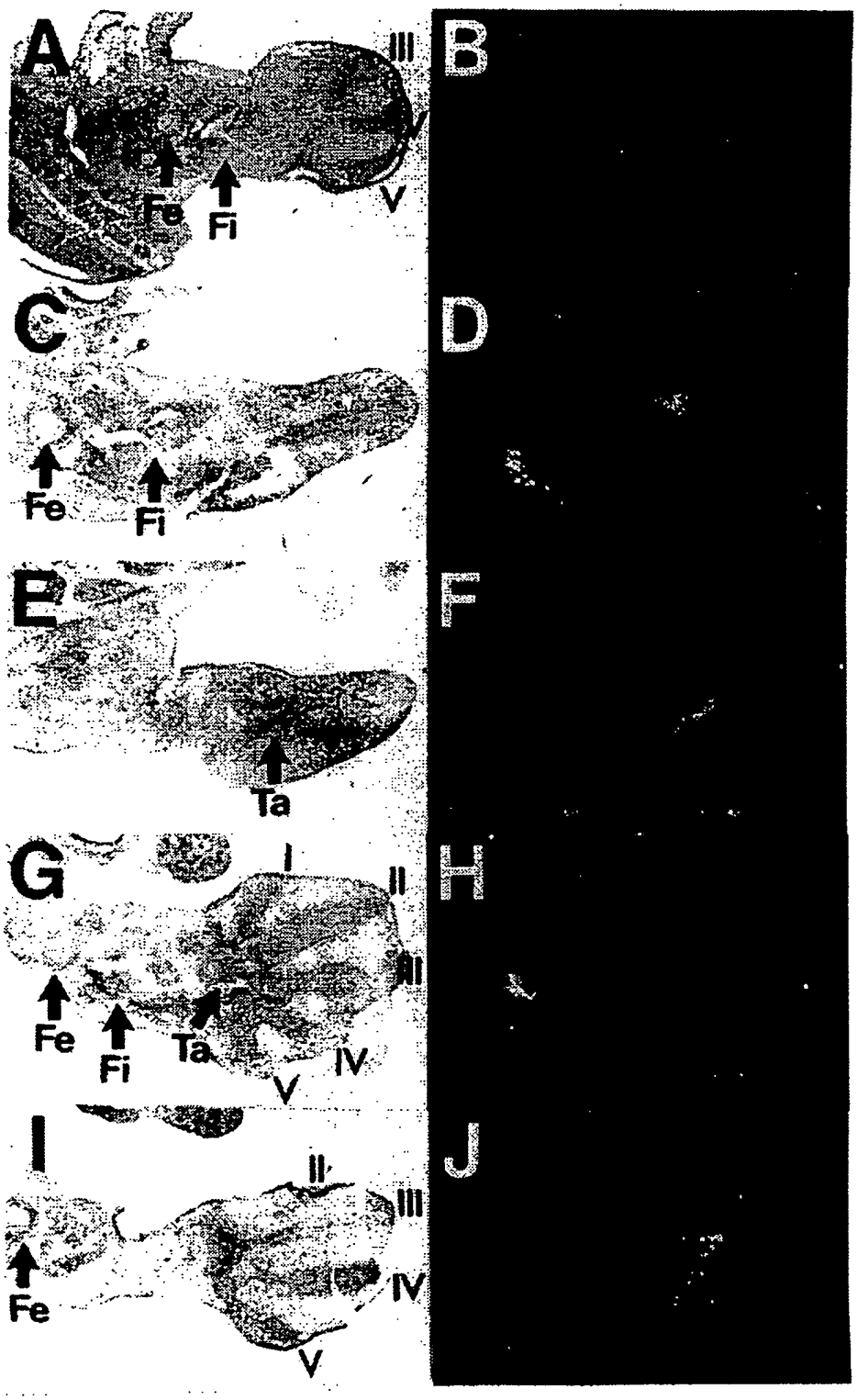
8．ＨＧＦが遺伝子組換えにより製造したものである請求の範囲第７項に記載の軟骨障害の治療法。

9．　軟骨障害が、変形性関節炎、軟骨形成異常症、骨折の治癒及び修復、外傷による関節軟骨及び関節円板の修復、急性化膿性関節炎、結核性関節炎、梅毒性関節炎、慢性関節リウマチ、リウマチ熱、全身性エリテマトーデス、変形性脊椎症、椎間板ヘルニア又は骨移植による修復である請求の範囲第７項又は第８項に記載の軟骨障害の治療法。

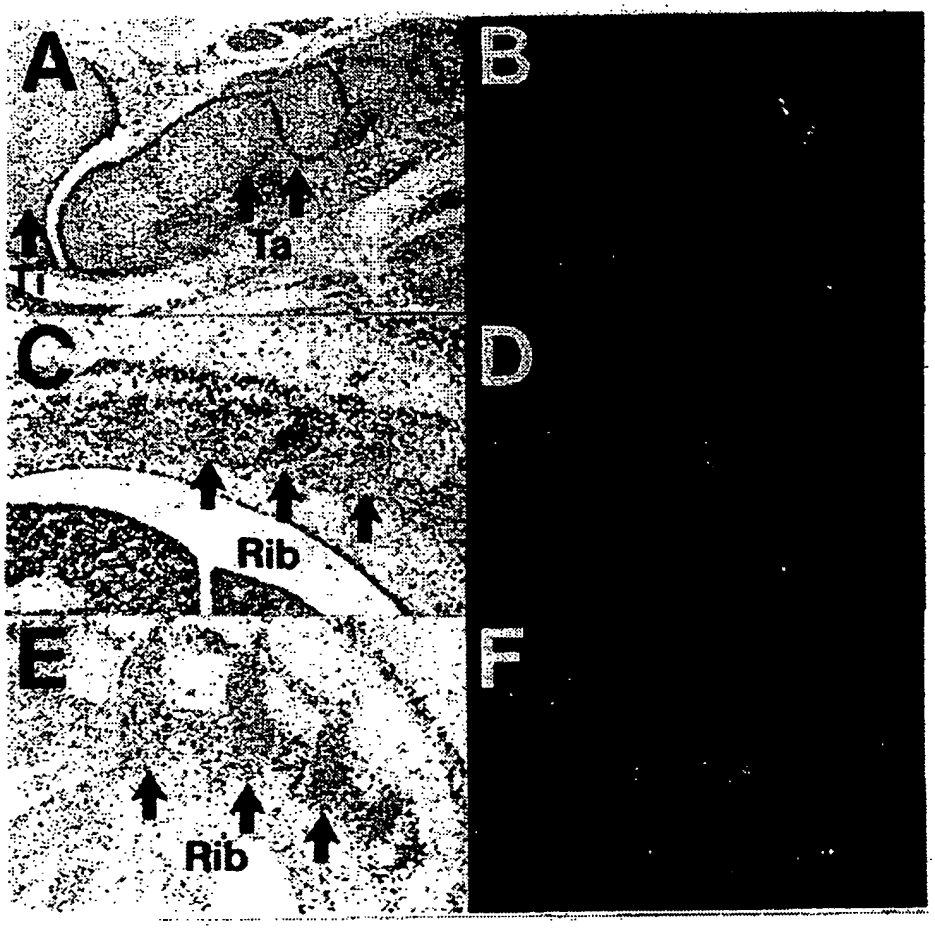
第 1 図



第 2 図

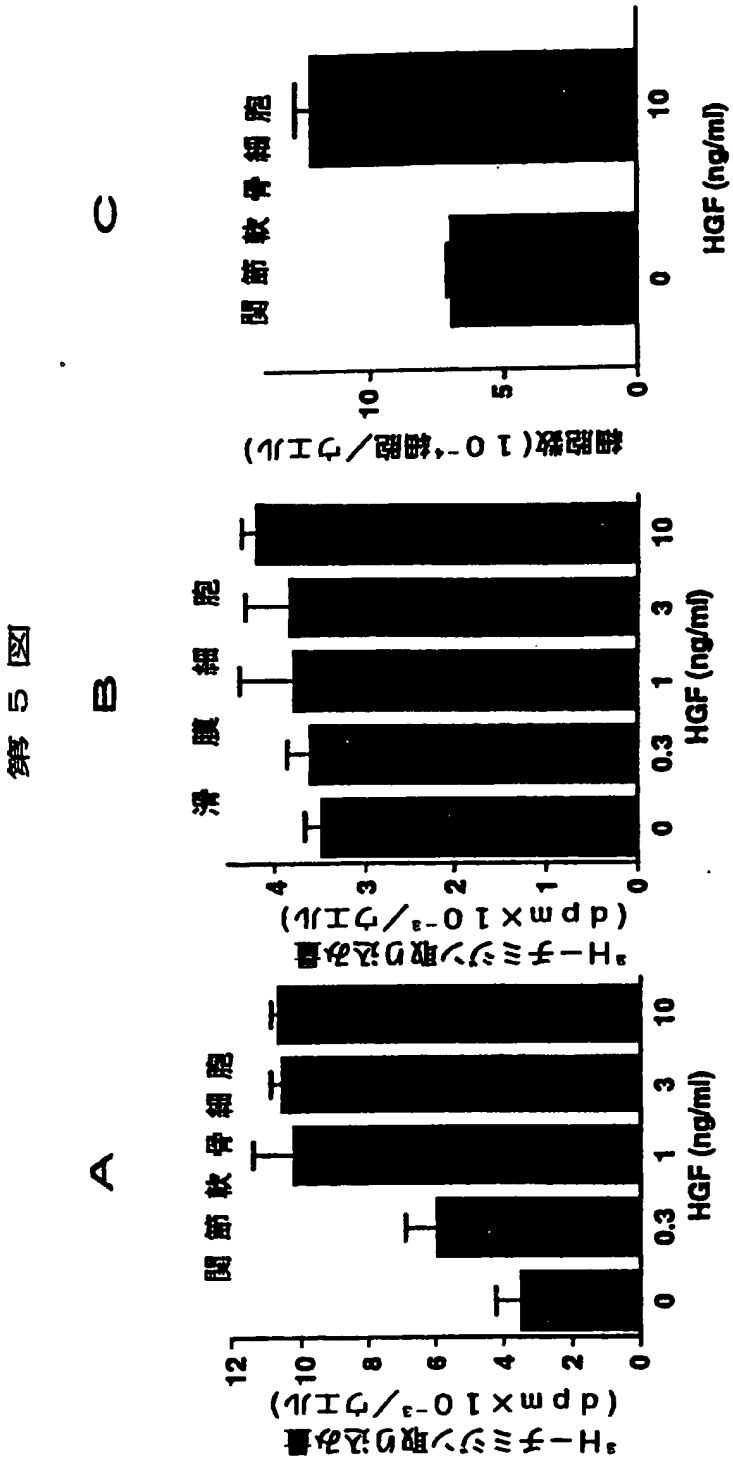


第 3 図

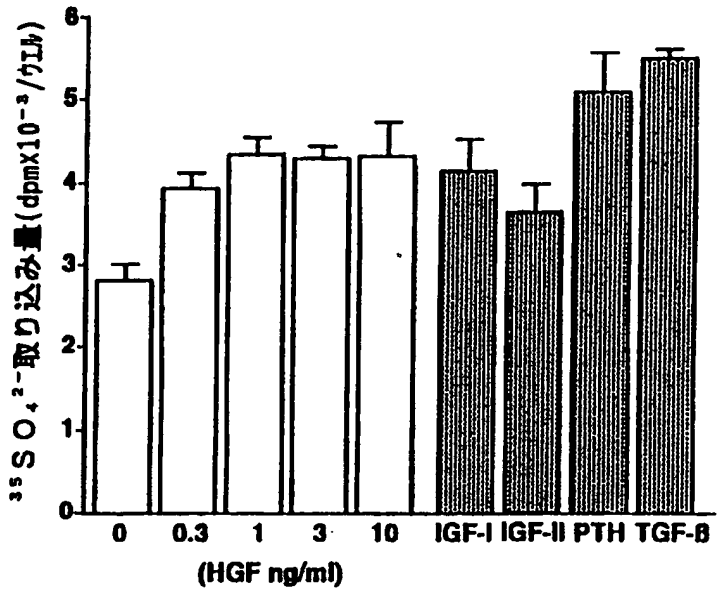


第 4 図

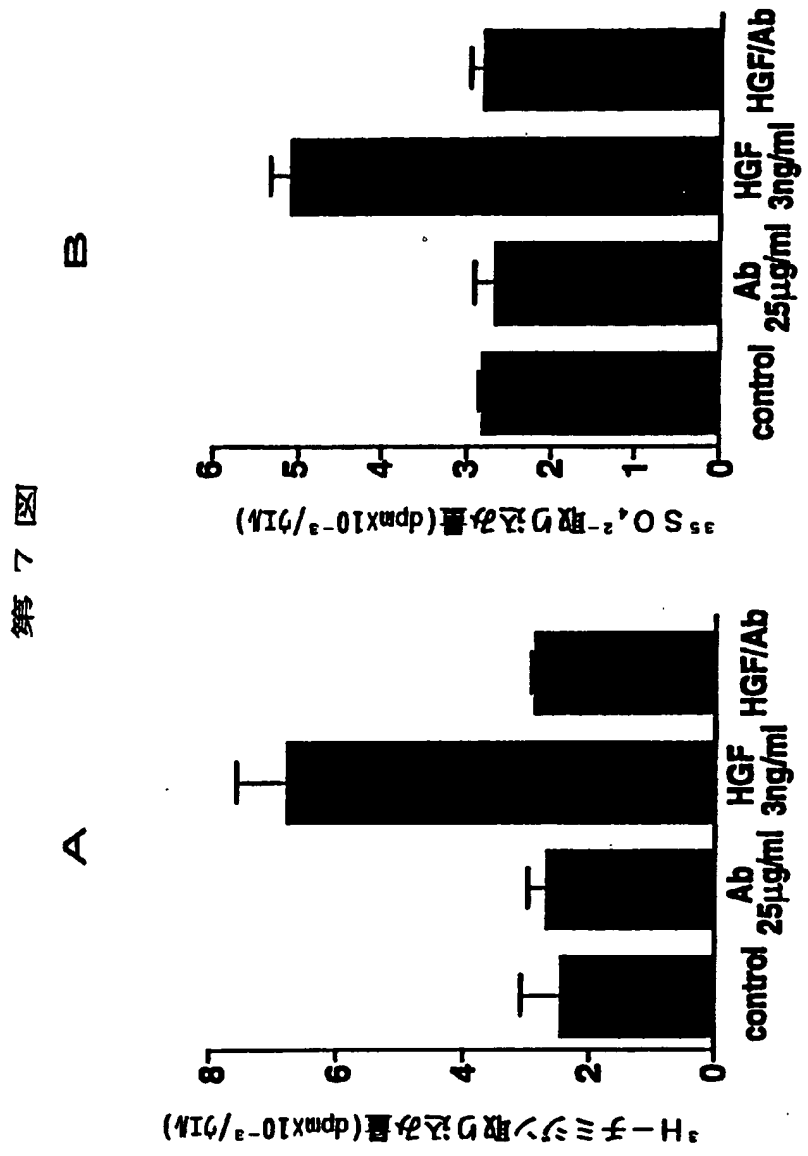
第５図


A
関節軟骨細胞
³H－チミジン取り込み量
(dpm×10⁻³／ウエル)
HGF (ng/ml)
B
滑膜細胞
³H－チミジン取り込み量
(dpm×10⁻³／ウエル)
HGF (ng/ml)
C
関節軟骨細胞
細胞数(10⁻⁴細胞／ウエル)
HGF (ng/ml)

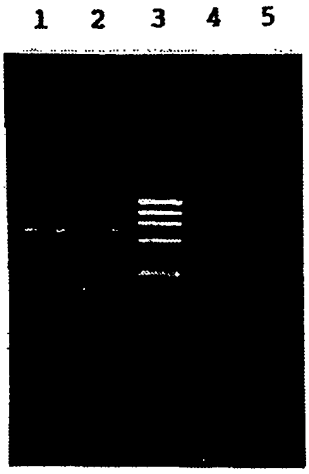
第 6 図


$^{35}SO_4^{2-}$取り込み量(dpmX10^{-3}/ウエル)
0
1
2
3
4
5
6
0
0.3
1
3
10
IGF-I
IGF-II
PTH
TGF-β
(HGF ng/ml)

第７図


A
B
control
Ab 25μg/ml
HGF 3ng/ml
HGF/Ab
0
2
4
6
8
^{3}H－チミジン取り込み量(dpmx10^{-3}/ウェル)
^{35}SO$_4^{2-}$取り込み量(dpmx10^{-3}/ウェル)

第８図



レーン

１：培養関節軟骨細胞（３５サイクル）

２：培養肋軟骨細胞（３５サイクル）

３：マーカー

４：関節軟骨組織（４０サイクル）

５：肋軟骨組織（４０サイクル）

INTERNATIONAL SEARCH REPORT

International application No.
PCT/JP95/00121

A. CLASSIFICATION OF SUBJECT MATTER

Int. Cl^6 A61K38/18//C07K14/475

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

Int. Cl^6 A61K38/18, C07K14/475

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	JP, A, 5-132426 (Takeda Chemical Industries, Ltd.), May 28, 1993 (28. 05. 93) & EP, A, 499242 & CA, A, 2061211	1 - 6
A	JP, A, 6-172207 (Toshikazu Nakamura and another), June 21, 1994 (21. 06. 94) (Family: none)	1 - 6
A	JP, A, 6-25010 (Toshikazu Nakamura) February 1, 1994 (01. 02. 94) (Family: none)	1 - 6

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:

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"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
April 10, 1995 (10. 04. 95)	May 2, 1995 (02. 05. 95)
Name and mailing address of the ISA/ Japanese Patent Office Facsimile No.	Authorized officer Telephone No.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/JP95/00121

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.: 7 - 9
because they relate to subject matter not required to be searched by this Authority, namely:

Claims 7 to 9 pertain to methods for treatment of the human or animal body by therapy, and thus relates to a subject matter which this International Searching Authority is not required, under the provisions of Articles 17(2)(a)(i) of the PCT and Rule 39.1(iv) of the Regulations under the PCT, to search.

2. [ ] Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

国際調査報告

国際出願 号 PCT/JP 95/00121

A. 発明の属する分野の分類（国際特許分類（IPC））

Int. Cl⁶ A61K38/18//C07K14/475

B. 調査を行った分野

調査を行った最小限資料（国際特許分類（IPC））

Int. Cl⁶ A61K38/18, C07K14/475

最小限資料以外の資料で調査を行った分野に含まれるもの

国際調査で使用した電子データベース（データベースの名称、調査に使用した用語）

C. 関連すると認められる文献

引用文献のカテゴリー*	引用文献名 及び一部の箇所が関連するときは、その関連する箇所の表示	関連する請求の範囲の番号
A	JP, A, 5-132426（武田薬品工業株式会社）, 28. 5月. 1993（28. 05. 93） &EP, A, 499242&CA, A, 2061211	1-6
A	JP, A, 6-172207（中村敏一 他）, 21. 6月. 1994（21. 06. 94）（ファミリーなし）	1-6
A	JP, A, 6-25010（中村敏一）, 1. 2月. 1994（01. 02. 94）（ファミリーなし）	1-6

☐ C欄の続きにも文献が列挙されている。

☐ パテントファミリーに関する別紙を参照。

* 引用文献のカテゴリー
「A」 に関連のある文献ではなく、一般的技術水準を示すもの
「E」 先行文献ではあるが、国際出願日以後に公表されたもの
「L」 優先権主張に疑義を提起する文献又は他の文献の発行日若しくは他の特別な理由を確立するために引用する文献（理由を付す）
「O」 口頭による開示、使用、展示等に言及する文献
「P」 国際出願日前で、かつ優先権の主張の基礎となる出願の日の後に公表された文献
「T」 国際出願日又は優先日後に公表された文献であって出願と矛盾するものではなく、発明の原理又は理論の理解のために引用するもの
「X」 特に関連のある文献であって、当該文献のみで発明の新規性又は進歩性がないと考えられるもの
「Y」 特に関連のある文献であって、当該文献と他の1以上の文献との、当業者にとって自明である組合せによって進歩性がないと考えられるもの
「&」 同一パテントファミリー文献

国際調査を完了した日	国際調査報告の発送日
10. 04. 95	02.05.95

名称及びあて先	特許庁審査官（権限のある職員）	
日本国特許庁（ISA/JP） 郵便番号100 東京都千代田区霞が関三丁目4番3号	松浦新司	4C 8314
	電話 号 03-3581-1101 内線 3452	

様式PCT/ISA/210（第2ページ）（1992年7月）

国際調査報告

国際出願番号 PCT/JP 95/00121

第Ⅰ欄 請求の範囲の一部の調査ができないときの意見（第1ページの1の続き）

法第8条第3項（PCT17条(2)(a)）の規定により、この国際調査報告は次の理由により請求の範囲の一部について作成しなかった。

1. ☑ 請求の範囲 7－9 は、この国際調査機関が調査をすることを要しない対象に係るものである。
つまり、

請求の範囲7－9は治療による人体又は動物の体の処置方法に関するものであって、PCT17条(2)(a)(i)及びPCT規則39.1(iv)の規定によりこの国際調査機関が調査をすることを要しない対象に係るものである。

2. ☐ 請求の範囲＿＿＿＿＿＿＿＿は、有意義な国際調査をすることができる程度まで所定の要件を満たしていない国際出願の部分に係るものである。つまり、

3. ☐ 請求の範囲＿＿＿＿＿＿＿＿は、従属請求の範囲であってPCT規則6.4（a）の第2文及び第3文の規定に従って記載されていない。

第Ⅱ欄 発明の単一性が欠如しているときの意見（第1ページの2の続き）

次に述べるようにこの国際出願に二以上の発明があるとこの国際調査機関は認めた。

1. ☐ 出願人が必要な追加調査手数料をすべて期間内に納付したので、この国際調査報告は、すべての調査可能な請求の範囲について作成した。

2. ☐ 追加調査手数料を要求するまでもなく、すべての調査可能な請求の範囲について調査することができたので、追加調査手数料の納付を求めなかった。

3. ☐ 出願人が必要な追加調査手数料を一部のみしか期間内に納付しなかったので、この国際調査報告は、手数料の納付のあった次の請求の範囲のみについて作成した。

4. ☐ 出願人が必要な追加調査手数料を期間内に納付しなかったので、この国際調査報告は、請求の範囲の最初に記載されている発明に係る次の請求の範囲について作成した。

追加調査手数料の異議の申立てに関する注意
☐ 追加調査手数料の納付と共に出願人から異議申立てがあった。
☐ 追加調査手数料の納付と共に出願人から異議申立てがなかった。